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ON THE CYTOLOGY OF TITYUS BAHIENSIS WITH SPECIAL REFERENCE TO MEIOTIC PROPHASE

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Received November 27, 1942

TITYUS BAHIENSIS, the common scorpion in the region of Piracicaba, São Paulo, Brazil, differs considerably with regard to form and number of chromosomes from related species described by other authors, as first stated by PIZA (1939a). Somatic divisions show six irregularly curved chromosomes; in the meiosis of spermatocytes three tetrads are easily distinguished, all chromatids being rod shaped, without showing signs of chiasmas, of constrictions, or any specific attachment region.

The most interesting feature so far reported is the parallel movement of the chromosomes or chromatid pairs during the first anaphase. This parallel movement in Tityus seems to be the same as described first by SCHRADER (1935) for some Hemiptera (see GEITLER 1938 for further references). PIZA (1939b) has formulated far reaching speculations on this subject with which we cannot agree.

The description of meiotic prophase as given by PIZA (1939a) is very incomplete. He describes very briefly leptotene, zygotene, and early pachytene stages, without corresponding illustrations. In the description of pachynema PIZA (1939a, p. 256) writes that "the nucleus seems to be occupied by extensive filaments of very clear chromomeric structure which describe many and large spirals, strongly accentuated in the point of separation of the respective segments." It is not clear to what structure the term "segment" refers. Later prophase stages were not observed in the three males studied by PIZA. He assumes that in late pachynema the tetrads condense and become regular in outline, passing thus into metaphase (fig. 3, *l.c.*). Finally PIZA declares categorically that "nothing exists which could be compared to a true chiasma" (*l.c.*, p. 257). Here again we encounter some difficulty in interpreting the statement without a clear definition of what the author calls a "true chiasma" (*quiasma verdadeiro*). Figures d and f (plate 1, *l.c.*), which show photomicrographs of a chromosome arrangement in metaphase with a reciprocal translocation, are described as "chiasmas between two bivalents caused by reciprocal translocations."

We could not find any further observations on the prophase in the later papers (PIZA 1939b, 1940, 1941).

The first difficulty in our study of this animal consisted in the relatively small number of males to be encountered. In several localities no male was found at all among several hundreds of females, and it has not yet been possible to decide about the method of reproduction in these cases. In other localities, all in the vicinity of the same town, males were found, though always in smaller number than females. Small individuals have generally very few cells in meiotic

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division and these are often all in the same phase, while larger males, which are rarer still, show a higher rate of division.

While leptonema and metaphase are frequently encountered, the intermediate stages are rare, and this paper is based essentially on the observations of about half a dozen males within some 30 studied. But whenever some phases were found in an animal, they were then very frequent. All possible care was taken, by observing the general characters of the cell, of the spindle, of the chromosome structure and size, to be certain that the different stages observed were arranged in the proper sequence and that no essential phases were overlooked.

The photomicrographs which accompany this paper were taken from aceto-carmine smears which gave the clearest pictures. In order to have all important structures more or less in one plane for photography, intact cells were flattened by slight pressure on the cover glass.

Leptonema, while frequently encountered, is impossible to analyze in detail, owing to the extreme length of the six fine threads which are densely interwoven. Zygotene pairing proceeds normally.

Figure 1 shows two cells in which pairing seems to be completed. In at least one of these, as shown by the arrow, the three pachytene pairs are visible. As is the rule in meiosis, three processes follow early pachynema: contraction by coiling or otherwise of the chromonemata, separation and consequent visibility of the four constituent chromatids, and the opening out of loops during diplonema. All these processes are clearly visible in Tityus (fig. 2–4), but diplonema leads to a complete separation of chromatid pairs. This may be seen, for instance, in the cell of figure 2 indicated by an arrow. One tetrad cannot be analyzed, the second tetrad has a typical diplotene structure with five loops in alternating planes, while the third tetrad is completely separated into two chromatid pairs.

Another case of opening out of loops is seen in figure 4. Here one tetrad is already completely separated into chromatid pairs, another opens up in the middle region, both ends still being closely paired, and the third tetrad seems united only in one point at a distance about one-fourth of the total length from its end. The chromatid structure is here especially clearly visible.

Finally at the end of diplonema, we encounter six double threads in each cell (fig. 5) showing to some degree a tendency to associate in three pairs, but without any sign of the preceding actual and intimate pairing. At this stage, the shortening of the chromatid pairs is already considerable, but has not quite reached its maximum.

We cannot say whether or not crossing over occurs at the points where diplotene loops change their planes and which conventionally are called chiasmas (see fig. 3). A genetical verification of the occurrence of crossing over or its absence is at present impossible in Tityus since no mendelian characters are known and since breeding experiments with this highly dangerous and poisonous animal do not seem advisable. Thus it is not possible in Tityus, where the number of seeming chiasmata is reduced between diplonema and metaphase,

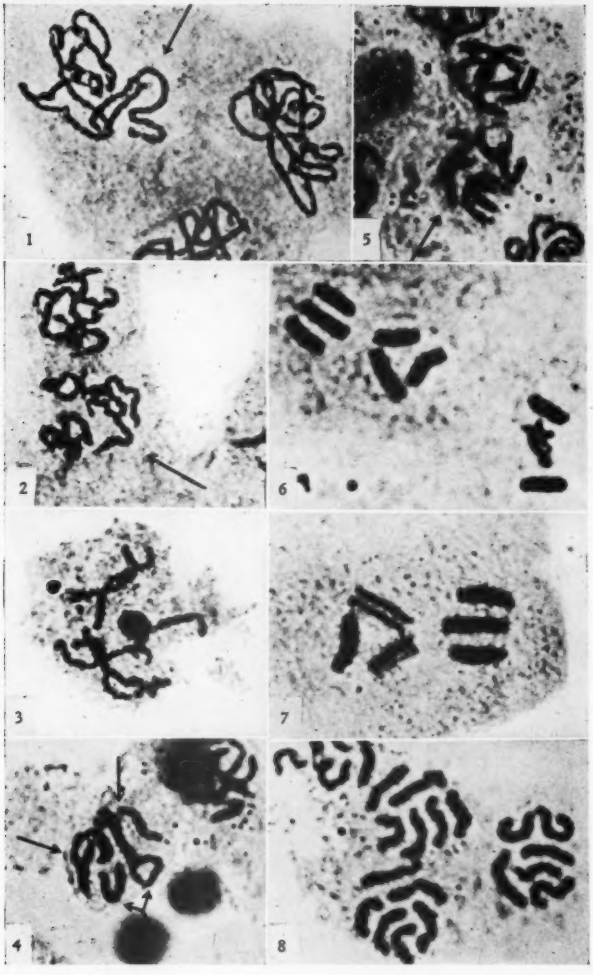

PLATE I

Photomicrographs of aceto-carmine smears; magnification 3000×.

FIGURE 1.—Pachynema.
FIGURES 2 to 4.—Successive stages in diplonema.
FIGURE 5.—Late diplonema, showing separation of homologues.
FIGURE 6.—Early metaphase.
FIGURE 7.—Late metaphase.
FIGURE 8.—Spermatogonial metaphase.

to say whether we are concerned with true chiasmata or to the unravelling of relationally coiled chromosomes.

No paraphase (pro-metaphase) stages have been found as yet, and the next stage encountered is metaphase. Taking into consideration the size and form of the chromatid pairs in the latest prophase and the earliest metaphase stages, it seems to us doubtful that any important stage is missing between those illustrated by figures 5 and 6. We may suppose that there is no pronounced diakinesis, since at the end of diplonema the chromosomes have already reached nearly the degree of contraction characteristic of metaphase. Of course there must exist a paraphase (pro-metaphase) during which the chromosomes or chromatid pairs move not only to the equatorial region, but begin or even complete their second pairing. We find invariably at metaphase three tetrads with chromosomes parallely paired, which may or may not show an intimate association at both ends, imitating thus terminalized chiasmas. No loops may be seen, only occasionally an unravelling of a chromatid spiral as in the lower right corner of figure 6. In some instances the more or less unravelled chromatid, only loosely and irregularly coiled, may be seen very clearly. From the general appearance and its not too frequent occurrence we feel convinced that this unravelling is of no especial significance and may be a "vital artefact" or may be caused by the fixative. More or less complete unravelling of chromatid coils is not rare in organisms with large chromosomes such as Hyacinthus, Rhoeo, and Tradescantia. Similar structures may be seen in the half schematical drawings by PIZA (1939a, fig. 5b, c, d), who offers no definite explanation except to reject the idea that they may be caused by an action of the spindle fibers. Later metaphases in polar views (fig. 7) show the chromatids well separated, strictly parallel, except for small terminal portions in some cases. Side views, which are difficult to photograph, show clearly and equidistant the ends of all four chromatids of each tetrad. This aspect is very similar to the schematical drawing of HUGHES-SCHRADER and RIS (1941, fig. 3), except that in Tityus there are not two, but three groups of four chromatids, each corresponding to a tetrad.

Thus, there can hardly be any doubt that two types of pairing occur: (1) normal zygotene pairing which subsequently disappears during diplonema and (2) metaphase pairing. Metaphase pairing has evidently no relation to the zygotene pairing. As a tentative and provisional hypothesis we may assume that it is caused by forces similar to those which cause somatic pairing, though the latter is never so intimate as in the present metaphases. It is difficult to understand how the four chromatids of each tetrad remain so closely parallel and equidistant during a stage which seems to persist during a relatively long time, judging by the frequency with which these metaphases are found. No connecting substance can be seen in the preparations, and it is useless to speculate about a responsible "force."

In order to test whether somatic pairing normally occurs, we studied spermatogonial divisions. There is some indication that the six chromosomes, all of which are of equal size and form, have a certain tendency to associate loosely

into three somatic pairs (fig. 8). More conclusive eivdence may be obtained in the future from individuals with unequal chromosomes due to translocations and chromosome attachments.

DISCUSSION

The two most important observations reported in this paper may be summarized in the following form.

Normal prophase pairing takes place in zygonema, but this association is followed by the complete separation of pairs of chromatids at a stage corresponding to diplonema.

A special kind of metaphase pairing is regularly found. Here four homologous chromatids, each well defined, are strictly parallel but equidistantly separated with the exception of some time during which the ends are in contact.

It seems interesting briefly to compare these findings with observations of other authors. Evidently we may distinguish: (1) Pairing in consequence of the attraction between homologous chromosome regions which leads to complete pairing in zygonema and to approximation and parallel arrangement in somatic pairing such as found in Diptera. (2) Pairing due to the attraction of ends of homologous chromosomes or chromatids as described by WILSON (1905) in Hemiptera, by REUTER (1930) for Alydus, and by SCHRADER (1940a) for Rhytidolomia. Where the so called "secondary pairing" should be included remains doubtful.

The parallel pairing during metaphase reported above and especially the cases of reciprocal translocations described by PIZA leave no doubt that metaphase pairing in Tityus is the result of the attractions between homologous regions and not of the whole chromosomes, nor is it due to only an end-to-end attraction. Judging by the frequency with which metaphases are encountered, the conclusion is justified that this stage is of considerable duration.

Prophase behavior in Tityus, with normal pairing at zygonema and complete separation at diplonema in consequence of the opening of all "chiasmas," is well in line with the hypotheses proposed by DARLINGTON (1937) with reference to pairing of meiotic chromosomes, but this is not true of the metaphase pairing. Except for the temporary union of both ends, homologous chromatids remain parallel and equidistant during a considerable time.

While normal zygotene pairing and somatic pairing as in Diptera are of the same nature in that they result from the "attraction" of homologous chromosome regions, there remains an essential difference as well: the presence or formation of chiasmas in the first and its absence in the other. Even though somatic crossing over has been described (STERN 1939), nothing has been reported as yet about a regular occurrence of anything which may correspond to chiasma formation in mitosis. It seems that the same two forms of pairing are found in Tityus, but both during meiosis. The zygotene pairing is completely normal, and typical chiasmas appear in diplotene. Metaphase pairing is much more complete and intimate than somatic pairing in Diptera, but in both cases no chiasmas are formed.

No reference has been made so far to the separation during anaphase, since

this phase has been only rarely observed by us. We had the opportunity of seeing most of Professor PIZA's slides but did not see definitive proof of the existence of two terminal spindle fibers per chromosome as claimed by him. PIZA's terminal spindle fibers may correspond actually to shadow planes connecting the chromosomes over their whole length with the poles of the spindle. In his figure 6D (PIZA 1941) one chromosome pair only is seen, and the other two might be arranged behind, as in his figure 5A, with the shadows actually produced by them. But in general it is always difficult to interpret properly the shadows which appear in the spindle after fixation and to determine to what extent they are only artefacts. While we cannot follow the deductions made by PIZA with regard to the mechanics of the anaphasic movement in Tityus, we are in accord with the interpretation given by HUGHES-SCHRADER and RIS (1941) for the apparently similar case of Steatococcus. Thus we do not find a decisive observation which proves the hypothesis of the bipolarity of chromosomes in Tityus, while on the other hand it is equally impossible to disprove it.

In a recent publication PIZA (1942) assumes that the chromosomes possess also a dorsoventrality and that "the spindle poles attract the ventral side of the chromosomes and repel the dorsal" (p. 157). Since it seems that the meiotic chromosomes of Tityus possess internal chromatid spirals at metaphase, a complicated twisting must be assumed if always ventral sides would be attracted by the poles. But since PIZA does not offer any decisive basis for his deduction and since no structural elements can be seen in Tityus which permit a distinction between dorsal and ventral sides, we shall not enter into a more detailed discussion of this point nor analyze the relation of PIZA's concepts to those of other authors.

The hypothesis of PIZA that chromosome pairing is due to an attraction between whole chromosomes and not to an attraction of homologous regions or loci finds no support in Tityus. In fact, the complete pairing in the reciprocal translocations described by PIZA seems rather to point against his hypothesis.

SUMMARY

A description of prophase and metaphase in *Tityus bahiensis* is given. As reported by PIZA, this species has six chromosomes and three tetrads in mitosis and meiosis, respectively.

Prophase behavior seems perfectly normal with pairing occurring during zygonema, a typical pachynema, followed by diplonema, Loops in alternating planes may be seen in the later phase, separated by "chiasmas." Finally chromatid pairs become completely separated.

No typical diakinesis was observed, and no clear paraphase could be found.

In metaphase the chromatid pairs are again paired, forming three tetrads per cell in normal males. This second pairing is presumably the result of an attraction between homologous chromatid regions. The chromatids may touch each other at their ends. But nothing which corresponds to chiasmas has been observed. Thus this metaphase pairing seems similar in nature to the somatic metaphase pairing in Diptera.

During anaphase the chromatid pairs remain at first strictly parallel, and no definitive attachment regions may be observed. While rejecting the various speculations of PIZA on the mechanics of this movement, we agree with the suggestion of HUGHES-SCHRADER and RIS (1941) that a diffuse attachment region extends over the whole length of each chromatid.

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AN AMPHIDIPLOID IN THE F_1 GENERATION FROM THE CROSS OENOTHERA FRANCISCANA×OENOTHERA BIENNIS, AND ITS PROGENY*

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Received January 19, 1943

CERTAIN peculiarities of the cross *Oenothera franciscana* Bartlett×*Oenothera biennis* L. led me to grow in recent years some large F_1 families in one of which there appeared a tetraploid plant the cytology and genetical behavior of which showed it to be an amphidiploid. This plant and its progeny will be the subject of this paper.

The cross *franciscana*×*biennis* from parents out of long inbred lines produces in the F_1 a progeny chiefly of light green, weak seedlings only a few of which live to develop small pale rosettes and these unable to live in the field. This was first recorded by DAVIS (1914, p. 189) and has been noted by later workers who have grown small families of the cross. But in contrast to the large progeny of light green, weak plants that die, there is a small group of dark green, strong seedlings which produce vigorous plants that come to flower in the field. The contrast between young rosettes of about the same age is shown in the text figure 1; above are the parent types, *franciscana* at the left and *biennis* at the right; below are the two types of F_1 hybrids.

Combined data from three large cultures (35.71, 36.71, 36.77) with a germination of about 95 percent gave 3672 pale green seedlings and rosettes almost all of which died rapidly in pans and pots and none of which survived in the field or when given special attention in the greenhouse. There were 44 plants, dark green and strong, all of which matured, and among these were three triploids and the amphidiploid.

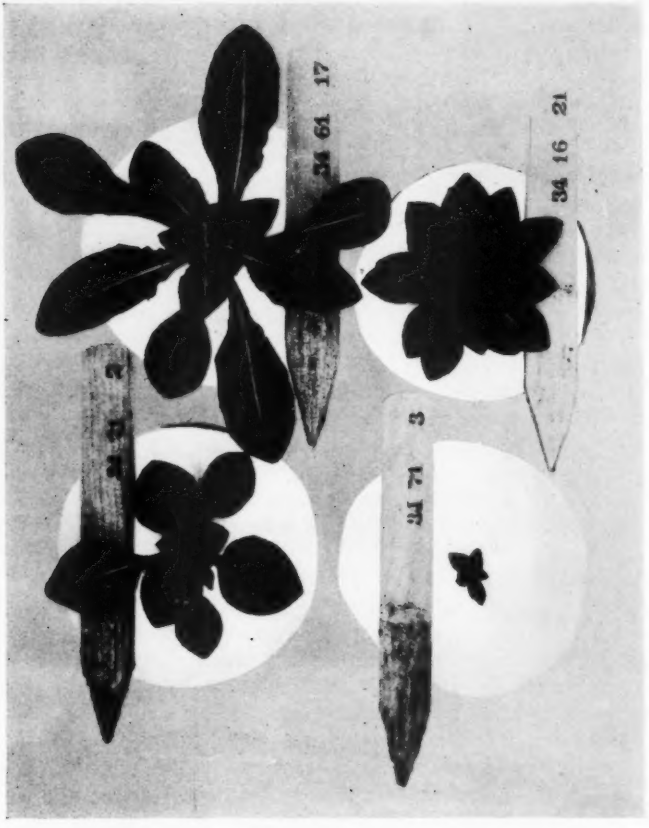
The three triploids differed from the other plants of the dark green type in greater vigor, larger leaves, absence of red spots on the rosette leaves, and in green bud cones. Of the pollen scarcely 10 percent of the grains seemed well developed, and these were probably infertile, since the plants produced no seed after numerous selfings, although forming large capsules when open pollinated. Cytological studies of meiosis in pollen mother cells of two plants showed that irregular distribution of the 21 chromosomes (10–11, 9–12, 8–13) characteristic of Oenothera triploids, and also showed frequent elimination of chromosomes through lagging on the spindle.

The amphidiploid was distinguished markedly from the other dark green plants in the following respects:

	Amphidiploid	Dark green plants
Mature rosette	5 dm broad. Leaves 25 cm long, 7–8 cm broad, much thicker.	3.5 dm broad. Leaves 18 cm long, 5–6 cm broad.

* The cost of the accompanying halftone illustrations is borne by the Galton and Mendel Memorial Fund.

[1] Cytological studies on Oenothera. VI. Papers from the Department of Botany, UNIVERSITY OF MICHIGAN, No. 712.



Text figure 1. *franciscana* upper left. *biennis* upper right.
Below, the two types of F_1 hybrids.

	Amphidiploid	Dark green plants
Mature plant	Central shoot 4.5 dm. Leaves at base 15 cm long, 5.5 cm broad, much thicker.	Central shoot 7–9 dm. Leaves at base 13 cm long, 4.5 cm broad.
Petals	27 mm long.	23 mm long.
Pollen grains	30 percent good, larger, 4-lobed, an occasional 3-lobed grain.	35–50 percent good, 3-lobed, an occasional 4-lobed grain.
Capsules	22–25 mm long, much stouter.	18–20 mm long, medium stout.
Chromosomes	N = 14.	N = 7.

The amphidiploid then differs from the dark green type in having larger and thicker leaves, larger flowers, longer and stouter capsules, and larger pollen grains generally 4-lobed.

The seeds were germinated in Petri dishes over wet filter paper. The cytological material was fixed in Karpechenko-Navashin with three changes during eight hours, washed over night, carried through a long series of alcohols, and stained with crystal violet after the method of Gram.

CYTOLOGY OF THE DARK GREEN DIPLOID PLANTS, F_1 FRANCISCANA × BIENNIS

Studies on two diploid plants (35.71–18 and 35.71–29) were in essential agreement with the prophecy of CLELAND (1932, p. 596) that the dark green hybrids from the cross *franciscana* × *biennis* would present the chromosomes at diakinesis in the configuration of a circle of ten and two pairs. I have not observed any closed circles of ten chromosomes but long chains are characteristic, sometimes a single one of ten chromosomes (fig. 1), but more frequently two or more chains of smaller numbers as in figure 2. The two pairs are very clear. The chains may still be found at metaphase of the first meiotic division. There are numerous irregularities of chromosome distribution during meiosis, responsible for at least part of the high degree of pollen abortion.

CYTOLOGY OF THE AMPHIDIPLOID

On the assumption that the amphidiploid arose from the diploid through somatic doubling of the chromosomes (see DISCUSSION) the 20 daughter chromosomes derived from the circle of ten or from equivalent chains would naturally tend to pair at diakinesis. However, where so large a number is concerned a failure of mates to find one another would be expected to lead to some groupings as trisomes, and longer chains or circles might be present. The four chromosomes derived from the doubling of each of the two pairs might give pairs at diakinesis or they might associate end to end to form circles or chains of four chromosomes. Therefore, apart from irregularities leading to trisomes, there might appear in the amphidiploid at diakinesis 14 pairs, or pairs and circles or chains of four or more, or no pairs but only circles or chains of four or more.

Figure 3 shows an example of diakinesis in which pairing seems to be proceeding in a regular manner, whereas in figure 4 chain arrangements are present that are confusing and might result in irregularities of meiosis. An illustration of all pairing at metaphase of the first meiotic division is given in figure 5; examples of such regularity were not easily found. Much more frequent were irregularities such as are shown in figure 6 where chains of three chromosomes are shown. Sometimes chains of four will be present as in figure 7, and such chains might consist of the four derivatives from the doubling of the pairs in the hybrid, or they might arise from the derivatives of the doubling of any two adjacent chromosomes in a chain.

Observations on the split chromosomes of interkinesis in the amphidiploid quickly established irregularities of chromosome count and distribution in the first meiotic division. Examples of regular distribution are not common. Segregation of chromosomes 15–13 were found. There is much lagging of chromosomes during the first division with the result that nuclei with 13 chromosomes are frequently formed (fig. 8, 9). In figure 9 all the chromosomes are accounted

for by a distribution of 13 with one, and 11 associated with three. It became established through plants in the generation from the amphidiploid selfed that some at least of the gametes with 13 chromosomes are functional. The irregularities of chromosome distribution must play a large part in the explanation of the high degree of pollen abortion in the amphidiploid.

In summary it should be emphasized that this amphidiploid did not present a settled behavior of all pairing on the part of the chromosomes at diakinesis. On the contrary, there was much irregularity in the process of chromosome segregation during meiosis. Accounts of amphidiploids have frequently assumed that these plants even from hybrids would breed true because the double set of chromosomes would permit a regular pairing between homologues. It will be noted that here is an amphidiploid Oenothera hybrid in which the pairing is far from regular with the result that the plant does not breed true, as will appear in the accounts of later generations.

A GENERATION FROM THE SELFED AMPHIDIPLOID

Here follows the history of a progeny from the selfed amphidiploid (culture 37.103). There were 355 seedlings from 426 seeds (three capsules), a germination of 83.3 percent. Seedlings and young rosettes were vigorous and presented a wide range in the width of the thick leaves. A representative group of 175 plants was set in the field. Mature rosettes followed the lead of younger, ranging from large with leaves about 20 cm long and 8–10 cm broad to small with leaves about 10 cm long and 5 cm broad. There were 41 large-leaved rosettes, 16 small-leaved, and 46 rosettes with leaves intermediate in size; 72 rosettes died in the field.

Of the 103 rosettes that lived only 31 produced flowering shoots. These plants were grouped in three classes.

1. Large flowers. These were represented by 11 plants from large rosettes, petals 30–35 mm long. They were larger-flowered and generally larger plants than the amphidiploid parent.

2. Medium-sized flowers. These included 18 plants from rosettes of medium size, petals 20–25 mm long. Most of the plants were similar to the amphidiploid.

3. Small flowers. There were two plants from medium-sized rosettes with petals 10–15 mm long and short capsules 14–16 mm long.

None of the small-leaved rosettes produced flowering shoots. Good pollen was usually 4-lobed, but some grains were 3- or 5-lobed. In certain plants the good pollen was as high as 90 to 95 percent of the output, but there were generally many shriveled grains present and good pollen in much smaller proportions, ranging from 10 to 80 percent.

EXPLANATION OF PLATES

All figures were drawn with the aid of a camera lucida under the Zeiss apochromatic objective 1.5 (N.A. 1.5) primary magnification 120, in combination with the compensating ocular K20. The figures are reproduced as magnified at stage level—that is, 2400 diameters.

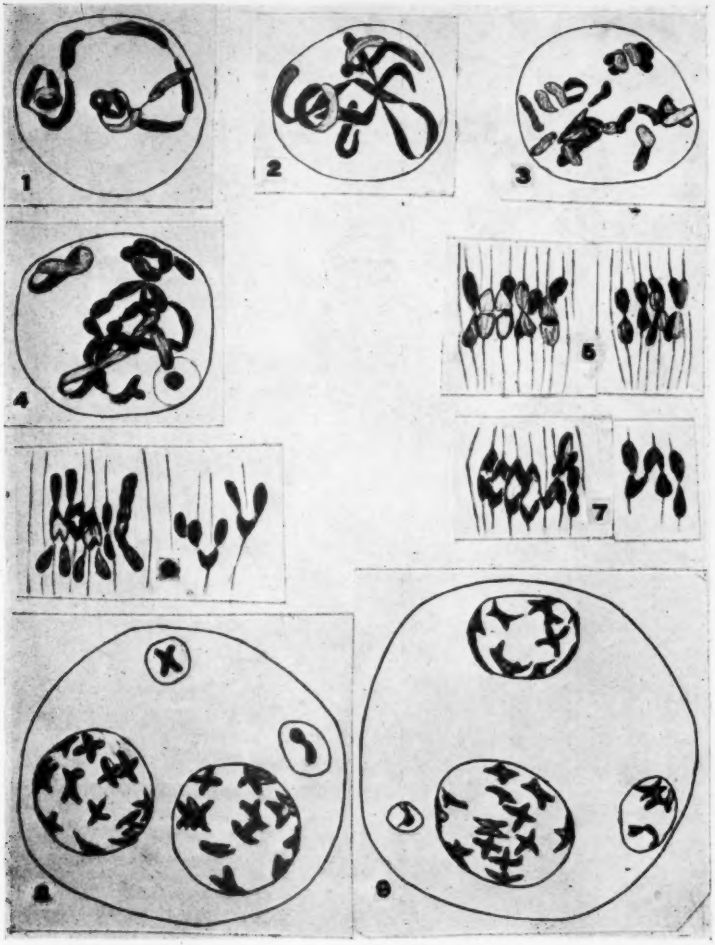


PLATE 1

FIGURE 1.—F_1 *Oenothera franciscana*×*O. biennis*. Diakinesis, chain of ten chromosomes and two pairs.

FIGURE 2.—F_1 *franciscana*×*biennis*. Diakinesis, shorter chains and two unpaired chromosomes, two pairs.

FIGURE 3.—Amphidiploid. Diakinesis, regular pairing apparently in progress.

FIGURE 4.—Amphidiploid. Diakinesis, chain arrangements present.

FIGURE 5.—Amphidiploid. First meiotic division, chromosomes all paired.

FIGURE 6.—Amphidiploid. First meiotic division, chains of three chromosomes shown.

FIGURE 7.—Amphidiploid. First meiotic division, chain of four chromosomes present.

FIGURE 8.—Amphidiploid. Interkinesis, two nuclei with 13 chromosomes the result of lagging of one pair.

FIGURE 9.—Amphidiploid. Interkinesis, irregular distribution to give two large nuclei with 13 and 11 chromosomes associated with two small nuclei of one and three chromosomes, respectively.

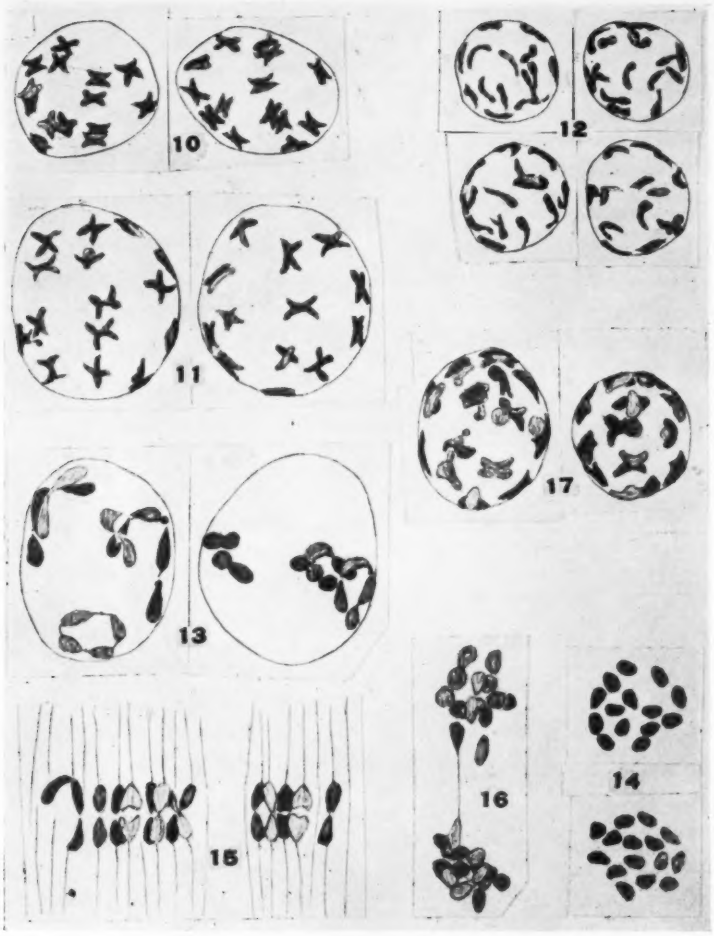


PLATE 2

FIGURE 10.—Plant No. 11. Interkinesis, the 28 chromosomes segregated 14–14.

FIGURE 11.—Plant No. 11. Interkinesis, the 28 chromosomes segregated 15–13.

FIGURE 12.—Plant No. 11. Nuclei from a tetrad, daughter chromosomes distributed by second meiotic division 14–14 and 15–13.

FIGURE 13.—Plant No. 34. Diakinesis, the 28 chromosomes in pairs, trisomes, and chains of four.

FIGURE 14.—Plant No. 45. The 29 split chromosomes at metaphase of the second meiotic division following a segregation 15–14.

FIGURE 15.—Plant No. 155. The 27 chromosomes at metaphase of the first meiotic division, 12 pairs and a trisome.

FIGURE 16.—Plant No. 155. Anaphase of first meiotic division, the unpaired chromosome backward in reaching a pole.

FIGURE 17.—Plant No. 155. Interkinesis, the 27 chromosomes segregated 14–13.

From this assemblage four plants were selected for selfing and for cytological studies. The plants were Nos. 11, 34, 45, and 155, and the findings were as below:

Plant No. 11. Large Flowers

Plant No. 11 with 28 chromosomes had large flowers (petals 30 mm), a large rosette, pollen 90 percent good, pollen grains 4-lobed with very few 3-lobed, germination from 379 seeds 31.1 percent.

The count of 28 chromosomes in Plant No. 11 was made in the two nuclei of interkinesis (fig. 10) from 20 cells, in five examples of the second division, and from two tetrads. There was much irregularity of distribution expressed in trisomes and chains of four in the first division, the former resulting in the formation of nuclei with 13 chromosomes either through non-disjunction in the distribution 13–15 (fig. 11), or by the lagging of one chromosome. Stages of interkinesis with nuclei showing 13 chromosomes were noted in 19 cells. The distribution 15–13 was found in four examples of interkinesis, in one second division, and in two tetrads (fig. 12). Rarely a nucleus was found with 12 chromosomes.

A family (culture 38.51) was grown from seeds of plant No. 11 selfed. The 118 young rosettes showed a wide range from large broad-leaved to small narrow-leaved plants. The latter were weak, and only 93 rosettes could be set in the field. Only 40 plants produced flowering shoots, 33 large-flowered and similar to the parent, and seven with medium-sized flowers. The genetical behavior of the plant was very similar to that of its parent amphidiploid. Therefore, Plant No. 11, like its parent, proved to be very far from a stable amphidiploid.

Plant No. 34. Medium-sized Flowers

Plant No. 34, also with 28 chromosomes, had medium-sized flowers (petals 23 mm), large rosette, pollen 80 percent good, pollen grains 4-lobed with few 3-lobed, germination from 481 seeds 45.3 percent.

The presence of 28 chromosomes was established by counts from 15 cells with the two nuclei in interkinesis, from some polar views of second division, and from the nuclei of two tetrads. As in plant No. 11, there was much irregularity at diakinesis expressed in trisomes and chains of four (fig. 13), although surprisingly large numbers of pairs emerged at metaphase of the first division. Non-disjunction and lagging gave numerous examples of nuclei with 13 chromosomes.

The generation grown from plant No. 34 selfed (culture 38.52) came through very well and showed much less variation than that from plant No. 11. There were set in the field 211 plants out of 218 seedlings, and of these 187 plants flowered. The mature rosettes were fairly uniform and of medium size. The flowers ranged from medium size to large. This rather uniform progeny (except for flower size) was much more like that to be expected from an amphidiploid than the generations from the other plants studied.

Plant No. 45. Dwarf, Seed Sterile

Plant No. 45 was a dwarf with 29 chromosomes, central shoot 3 dm high, flowers medium-sized (petals 20 mm), sepals very hairy, pollen 40 percent good, pollen grains 4-lobed with few 3- or 5-lobed, no germination from 90 seeds.

The count of 29 chromosomes was made from eight examples of interkinesis showing the two nuclei with 14 and 15 chromosomes, respectively, from two cells with nuclei in metaphase of the second division (fig. 14) and in three tetrads. The distribution of the chromosomes at interkinesis was usually 14–15, but lagging frequently gave nuclei with 13 chromosomes. Seven other plants in the culture were similar in appearance to Plant No. 45.

Numerous self-pollinations were made, but only one small capsule developed with 90 seeds that failed to germinate. The seeds although fair-sized had very little contents.

Plant No. 155. Small Flowers

This 27 chromosome plant was characterized by small flowers (petals 10 mm), small leaves, medium-sized rosettes, pollen 60 percent good, pollen grains about half 4-lobed and half 3-lobed with a few 5-lobed, capsules small (14–16 mm long), germination from 124 seeds 59.9 percent.

The chromosome count of 27 was determined in two examples of first division (fig. 15, 16) in 14 nuclei in interkinesis (fig. 17) in two examples of second division and in three tetrads. The odd or unpaired chromosome is sometimes alone at the side of the equatorial plate in the first division, or it may be associated with a pair to form a trisome (fig. 15). It frequently moves less rapidly to the pole of the spindle (fig. 16), and sometimes fails to be included in a daughter nucleus. The distribution 13–14 (fig. 17) was common. A few nuclei of interkinesis were noted with 12 and 15 chromosomes the results of nondisjunction.

The plant set small capsules with few seeds and did not hold the capsules well. From 18 selfings only four capsules were collected producing a total of 124 seeds. Of these seeds 63 proved fertile and gave 60 rosettes that were set in the field. The rosettes at maturity were fairly uniform and of medium size, but the mature plants fell into groups distinguished by flower, leaf, and capsule size. There were nine plants like the parent, with small flowers, leaves, and capsules; 30 plants with medium-sized flowers and large capsules; nine plants with large flowers, large leaves, and large capsules; nine rosettes which failed to produce flowering shoots; three plants that died.

This is a segregation such as one might expect from the 27 chromosome parent. The small-flowered plants probably repeated the combination 13–14 of the parent and the larger plants with large flowers, leaves, and capsules, the 14–14 combination of the amphidiploid.

Some of the early studies on amphidiploids, analyzed by WINGE (1932), led to a high degree of confidence in their stability and fertility as "constant species hybrids." But as more examples have been investigated it has become evident that irregularities of chromosome distribution at meiosis are common

and that the pairing of sister chromosomes (following somatic doubling) may not take place as regularly as might be expected. Other conditions, discussed by GOODSPEED and BRADLEY (1942), also lead to inconstancy of amphidiploids, but irregularities of chromosome distribution probably cause the greatest disturbance. The amphidiploid from *franciscana*×*biennis* illustrates well that inconstancy which goes with irregularities in chromosome associations and distribution expressed in aneuploidy and forms of sterility.

DISCUSSION

We are dealing with a tetraploid in the F_1 of the cross *franciscana*×*biennis*, and there would seem to be only two possible ways by which it might have arisen. First, and much more probable, a doubling of the two parental sets of chromosomes may have taken place in the zygote or young embryo through the failure of one or more mitoses to effect the distribution of daughter chromosomes, thus establishing reorganized somatic nuclei with double sets of chromosomes. Second, possible but improbable, a chance diploid gamete of *biennis* might have met a chance diploid gamete of *franciscana*. The first suggestion follows the views of GATES (1909, 1911) on Oenothera and the hypothesis of WINGE (1932) on "constant species hybrids" for which much supporting evidence is known. The second possibility is unlikely, since triploids which show the presence and functioning of diploid gametes are rare in pure line cultures of *biennis* and *franciscana*. My observations on the origin of an amphidiploid in the F_1 of a cross add to the findings of RENNER (1933) who has reported five highly fertile and fairly constant tetraploids directly from crosses.

When amphidiploids come from a parent with chromosome complexes of one or more circles, it becomes a matter of interest to know to what extent such chain associations may appear at diakinesis. The *franciscana* parent of this amphidiploid was of a race with all pairing chromosomes, but the *biennis* parent had the complex of two circles of six and eight chromosomes, respectively. The dark green hybrid *franciscana*×*biennis* presents at diakinesis a chain or circle of ten chromosomes and two pairs. Although the material studied was limited, large circles were not found at diakinesis in the amphidiploid, at best only chains of three and four chromosomes.

SEITZ (1935) has published a large body of observations on chromosome arrangements in the prophases of the first meiotic division in tetraploids from *franciscana*, *biennis*, and *Lamarckiana*. The observed associations are shown in tables for a large number of nuclei. Important are his findings that the largest circles and chains contain no more chromosomes than would be expected from the configurations in the parent plants. His material of *franciscana gigas* was from a race of *franciscana* with a circle of four chromosomes and five pairs, and SEITZ found in the tetraploid circles or chains of eight chromosomes but never more than one in a single nucleus.

The material of *biennis gigas* studied by SEITZ presented most of the chromosomes in pairs or quadrivalents. However, since the chromosomes of *biennis* are in circles or chains of six and eight, such might occur in the tetraploid, and they were found. The persistence of some groups of six and eight was remark-

able, one nucleus showing two of each. Another nucleus had two chains of 12 and two pairs; this condition might arise through breaks in the circles at the same point and the union of free ends, accompanied by the splitting off of two pairs.

SEITZ reports for *Lamarckiana gigas* at diakinesis numerous pairs and less frequently quadrivalents in chains and circles, together with occasional single chromosomes, trisomes, and groups of more than four. Since the chromosome configuration of *Lamarckiana* is a circle of 12 and one pair, larger circles and chains than four might be expected in *gigas*, but they were rarely found. The failure to recover anything resembling the circle of 12 in *Lamarckiana* is an important point in this account. The larger circles of diploids are maintained with greater difficulty in tetraploid derivatives. The greater the number of chromosomes in the convolutions of a chain the greater will be the probability of breaks when related chromosomes in two chains find themselves in favorable positions to pair in diakinesis. Irregularities of chromosome distribution at meiosis leading to high pollen sterility have been reported for *Lamarckiana gigas* from the earliest accounts of its cytology (GATES 1909, 1911; DAVIS 1911; HOEPPENER and RENNER 1929). BOEDIJN (1924) described a *gigas-lata* with 29 chromosomes and this must assume the functioning of a 14+1 gamete.

It is well known that *Lamarckiana gigas* throws a varied progeny: narrower-leaved and smaller-flowered forms, some of them dwarfs, besides the broad-leaved large-flowered type that carries the line. There is thus close parallelism in both cytological and genetical behavior between *Lamarckiana gigas* and the amphidiploid described in this paper. But this parallelism would be the same whether *gigas* arose by somatic doubling or through the fusion of suitable diploid gametes. CLELAND (1929, p. 138) has reported tetraploid pollen mother cells in a loculus of *Lamarckiana* all in heterotypic metaphase; from such cells would be expected diploid pollen with two sets of *Lamarckiana* chromosomes. The triploids (semi-gigas) that occasionally appear in lines of *Lamarckiana* prove the functioning of diploid gametes, and extensive breeding from such triploids (highly sterile) out of *Lamarckiana* accompanied by detailed cytological studies might establish the origin of a tetraploid through the fusion of diploid gametes. It seems most improbable that dispermy has played an important part, if any, in the origin of triploids in Oenothera (DAVIS 1933, p. 294). However, the possibility that the original *gigas* plant came from *Lamarckiana* through somatic doubling can never be ruled out.

The position of GATES (1909) who early held that *Lamarckiana gigas* arose through somatic doubling of the chromosomes of *Lamarckiana* (a structural hybrid) has support in the findings of RENNER (1933) of five highly fertile and fairly constant tetraploids among the progeny of direct crosses, and in the appearance of this amphidiploid in the F_1 from the cross *franciscana*×*biennis*. These tetraploids are all from diploid ancestry. But this does not weaken as an hypothesis the position of STOMPS (1912) who proposed the view that the union of suitable diploid gametes from triploids would give tetraploids. Since triploids (*semi-gigas*) are known from many lines of Oenothera, it is neces-

sary only to secure sufficient genetical and cytological evidence to place this hypothesis in a strong position.

Such evidence for the fusion of diploid gametes came through the history of a tetraploid *franciscana* by way of a triploid in the F_2 of the cross *franciscana*×*franciscana sulfurea nana* (DAVIS 1933). This is not the place to repeat the details of this genetical history and the cytological behavior that accompanied it, but the following important points may be emphasized because of a postscript to RENNER'S paper of 1933. The 21 chromosomes of the triploid were believed to be in three homologous sets because they grouped themselves at diakinesis in trisomes. Segregation of 7–14 chromosomes (one set and two sets) took place; much irregularity in association and distribution of the chromosomes was responsible for a high degree of pollen sterility. The triploid gave a progeny of 42 *franciscana* plants (14 chromosomes), 44 various dwarfs, and three thick-leaved tall plants (probably all tetraploids), one of which was established to be a tetraploid. The evidence is very strong that the 42 *franciscana* plants came from the union of seven chromosome gametes and the tetraploid from the union of 14 chromosome gametes. RENNER, however, is not impressed by this performance and holds to the possibility of the union of gametes with chromosomes seven and 21, or of gametes with chromosomes of intermediate numbers. He thus suggests a 21 chromosome gamete of which Oenothera research gives no evidence, or the union of gametes of intermediate numbers which would greatly decrease the probabilities of a zygote carrying four sets of *franciscana* chromosomes and consequently breeding true as did this tetraploid.

It can be proposed that a *franciscana* zygote from the union of *franciscana* gametes out of the triploid might have produced the tetraploid through chromosome doubling. Against this possibility is the record of my selfed *franciscana* line which since 1913 has been carried through 21 complete generations with a total of 6946 *franciscana* plants (diploid), three triploids, and four haploids, but no tetraploids. The triploids came from seeds from small capsules, the result of experiments on sparse pollination (DAVIS 1937, p. 103, table 2). This experiment indicated that pollen tubes of *franciscana* carrying diploid gamete nuclei grow more slowly than the normal and have little or no chance to effect fertilization under the usual conditions of excess pollination. Supporting this record is that of a selfed line derived from a haploid selfed which, carried through six generations, gave a total of 3922 *franciscana* plants and three haploids, but no triploids or tetraploids (DAVIS 1937, p. 105).

It seems probable that in Oenothera there are two methods by which tetraploids may arise: (1) By somatic doubling, possible from any diploid line and most likely when the origin is from an immediate cross; (2) through the union of diploid gametes, probable when the origin is from a triploid. It has been the writer's good fortune to study material believed to illustrate both types of origin, the first as recorded in this paper and the second in the account of the tetraploid *franciscana* (DAVIS 1933).

SUMMARY

The cross *franciscana*×*biennis* gives a progeny mostly of plants pale green and weak which die rapidly as seedlings and small rosettes. There is a small class of dark green seedlings that produce large strong rosettes and mature plants. Combined data from three large cultures gave 3672 pale green seedlings and rosettes and 44 dark green plants among which were three triploids and the amphidiploid.

The amphidiploid differed from the dark green diploids in having larger and thicker leaves, larger flowers, longer and stouter capsules, and pollen grains mostly four-lobed.

The dark green diploids have a chromosome configuration of a circle or chain of ten chromosomes and two pairs, or the ten chromosomes may be grouped in two or more smaller chains.

The amphidiploid at diakinesis presented a wide range of chromosome groupings. Complete pairing was rare. Usually there was a mixture of pairs, trisomes, and chains of four chromosomes.

Examples of regular distribution of the 28 chromosomes of the amphidiploid by the first meiotic division were not very common. Segregations of 13 and 15 were found with other irregularities leading to much abortive pollen. Fertile gametes were formed carrying 13 and 15 chromosomes, since the amphidiploid threw plants with 27 and 29 chromosomes.

The first generation from the amphidiploid presented an unexpectedly diverse progeny: (a) 11 plants with flowers larger than the amphidiploid (test plant No. 11, 28 chromosomes), (b) ten plants with medium-sized flowers, similar to the amphidiploid (test plant No. 34, 28 chromosomes), (c) eight dwarfs with medium-sized flowers, the sterile seeds with very little content (test plant No. 45, 29 chromosomes), and (d) two small-flowered plants (test plant No. 155, 27 chromosomes).

Second generations from the four selected plants gave: (a) from plant No. 11 (28 chromosomes, large flowers) a wide range of progeny in leaf form and flower size, a behavior similar to that of the parent amphidiploid; (b) from plant No. 34 (28 chromosomes, medium-sized flowers) a fairly uniform progeny, such as one would like to obtain from an amphidiploid; (c) from plant No. 45 (29 chromosomes, a dwarf) no germination from 90 seeds; (c) from plant No. 155 (27 chromosomes, small flowers) nine plants like the parent, 39 plants with medium-sized flowers, nine large-flowered plants.

Thus, this amphidiploid in the irregularities of its breeding showed itself to be far from a "constant species hybrid."

It is held that tetraploids in Oenothera may arise and in material studied by DAVIS have arisen both by somatic doubling (most likely when from diploids) and through the union of diploid gametes (more probable when the origin is through triploids).

Origin by way of somatic doubling seems more probable when a tetraploid appears directly from a cross as in this amphidiploid from *franciscana*×*biennis* and the five tetraploids from crosses obtained by RENNER (1933).

Origin through the union of diploid gametes, as held by STOMPS, is very much more likely when the tetraploid is from a triploid. From such an origin the tetraploid *franciscana* is believed to have arisen, the genetics and cytology of which was described by DAVIS (1933).

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GENES AFFECTING TESTIS COLOR IN EPHESTIA KÜHNIELLA Z

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INTRODUCTION

THE testis color of *Ephestia kühniella* has been studied intensively in the *aa* and $a^k a^k$ races (CASPARI 1933). Wild type (a^+) animals have black eyes and brightly colored testes, whereas the testes of the *aa* (red-eyed) mutant are usually colorless, although they may contain slightly pigmented spiral lines. Mutant $a^k a^k$ animals are intermediate, having brown eyes and usually slightly colored testes. Within the *aa* and $a^k a^k$ strains, a high correlation was found between the depth of eye and testis coloration. This correlation was found to be due to concomitant variation under the influence of temperature and other unspecified factors. The concomitant variation under the influence of the genes of the *a* series was proved by transplantation experiments to be due to the presence in a^+ of a substance necessary for the formation of pigment in eyes and testes which was called a^+ substance and has since turned out to be kynurenin (BUTENANDT, WEIDEL, and BECKER 1940). This substance is reduced in amount or qualitatively changed in a^k, and further reduced or absent in *aa*.

The testis coloration of a^+ animals was not specially investigated in the above study (CASPARI 1933). The a^+ race was merely used as a standard phenotype to which the phenotypes corresponding to the two other genes were referred. The variability of a^+ testes was observed in the course of this investigation and roughly estimated, in order to show the consistent difference in testis color between a^+ and the lighter colored races, and in order to demonstrate the complete dominance of a^+ over a^k and *a*. It was observed, however, that two types of testis coloration occur among a^+a^+ animals, a brownish violet and a more reddish shade. The genetic basis of this difference has been investigated, and the results are presented in this paper.

MATERIALS AND METHODS

The strains of a^+a^+ Ephestia used were obtained through the courtesy of DR. P. W. WHITING. They had not been inbred in his laboratory, but had been kept in mass cultures, since they were used only for the feeding of Habrobracon. Furthermore, an *aa* strain was used in some experiments. This strain is identical with the light-eyed strain R_1 described in 1933 and has been maintained by brother-sister matings ever since.

The animals were reared in fingerbowls, four inches in diameter and one and

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one-half inches deep, and fed yellow granulated cornmeal. They were reared in a room whose temperature was kept above 24°C by means of a thermostatically controlled electric blower.

The testes were dissected out in 0.7 percent saline or in tap water, usually within 24 hours after hatching. Sometimes, especially if the animals had been used for breeding, it was necessary to dissect them later. Comparisons showed that the apparent quantity of the pigmentation changed during adult life, but the quality remained the same. The coloration of the testes was measured with Ostwald's Farbkörper as described previously (CASPARI 1933). In some instances, only the kind of pigment was noted.

RESULTS OF BREEDING EXPERIMENTS

Ephestia larvae have two testes situated dorsally in the fifth abdominal segment. The testes are kidney-shaped and divided into four follicles by follicle walls. They are unpigmented usually up to the larval instar before the last. About the time of the last larval molt pigment begins to appear first in the follicle walls. The amount of pigment increases through the last larval instar, spreading over the whole surface of the testis. Shortly before the pupation the testes fuse. After pupation, they become twisted around each other spirally, so that the follicle walls become distorted. Adult Ephestiae therefore have one round testis. The pigmentation is strongest in eight spiral lines corresponding to the follicle septa. The surface between these lines is more or less covered with irregular blotches of pigment, the strength of color depending on the amount of yellow background showing between the pigmented areas. Histologically, the pigment is distributed in heaps of fine granules throughout the follicle walls and the innermost layer of the testis sheath which is continuous with the follicle walls.

The pigment in the testis sheath of a^+ Ephestia may be of two kinds, either brownish purple or red. This difference had already been observed on German material in 1933, and colored pictures of both types were given (CASPARI 1933, fig. 2, d and e). The same two types were reencountered in the American material obtained from DR. WHITING. Through selective inbreeding, two strains were established which have bred true since for brown and red testis coloration for 14 and 15 generations, respectively.

The results of crosses between these two strains are represented in table 1. The two kinds of testis pigment can usually be distinguished easily and do not overlap. However, in specimens where the pigment is very faint, they cannot be distinguished with certainty. Such animals are classed as questionable in table 1.

Two animals in the inbred strains, one in each, showed the phenotype characteristic of the opposite stock. They are probably immigrants from other cultures. The F_1 testes are uniformly brown. Segregation in F_2 and in the backcross to the red testis strain agrees well with the expectations for a 3:1 and a 1:1 ratio, respectively, which would be 513 brown:171 red$\pm$11.33 for F_2 and 222.5 brown:222.5 red$\pm$10.57 for the backcross. The difference in color be-

tween these two stocks is therefore due to one pair of alleles, the gene for brown testis color (*Rt*) being dominant over its allele for red testes (*rt*). A symbol for the wild type condition has been avoided, since it is likely that both alleles have occurred independently in non-inbred German as well as American stocks. That at least the identical gene *rt* was present in the German stocks will be shown below.

THE NATURE OF THE PIGMENTS

The pigments depending on the genes *Rt* and *rt* seem to be qualitatively different from each other. This difference appears clearly in sections. Testes from both stocks were fixed in Bouin, embedded in paraffin, and observed unstained or after staining with Boehmer's hematoxylin. In such sections the

TABLE 1

Results of crosses between Ephestia strains with red and brown testes.

TYPE OF MATING	TESTIS COLOR OF OFFSPRING			
	brown	red	?	Total
brown×brown	860	1	3	864
red×red	1	1403	3	1407
red×brown (F_1)	101	—	—	101
brown×red (F_1)	111	—	1	112
F_1×brown	73	—	1	74
F_1×red	218	225	10	453
F_1×F_1	502	182	11	695

testis appears surrounded by a unicellular layer. The nuclei are relatively big, round or ovoid, and are distributed at quite regular distances. No cell boundaries could be observed. This layer of cells is bordered externally by a structureless membrane. Internally, a thicker structure divides this layer from the lumen of the testis. This internal sheath is continuous with the follicle septa, thin membranes which are somewhat enlarged at irregular intervals and contain oblong nuclei at these places. The pigment is distributed in irregular heaps of granules of different size in the internal testis sheath as well as in the follicle septa which are continuous with it. Especially in unstained sections it can be seen that the single pigment granules in *rt rt* testes are definitely red, whereas *Rt Rt* testes contain a sepia colored pigment.

No difference in chemical behavior between brown and red pigment has been found. Both are insoluble in water, alcohol, ether, chloroform, acetone and dilute acids. Both are dissolved and immediately destroyed in 1n NaOH and 1n NH_4OH. Both are dissolved in n/10 Na_2CO_3 and are stable for several days. Solutions of *Rt Rt* pigment have a distinct purple color, whereas the *rt rt* pigment appears faintly yellowish in solution. Both are precipitated at neutralization. They are distinct from melanins as indicated by their destruction in dilute alkalies. They do not seem to be identical with the eye pigment of

Ephestia (skotommin, BECKER 1939) since they are insoluble in acidified alcohols.

No evidence for pleiotropic action of the genes *Rt* and *rt* has been found. The color of the larval ocelli and of the adult brains is identical in both races, and the pigment of the eyes consists of sepia granules in both strains as observed in sections. The values obtained in F_2 and in the backcross of the heterozygote to *rt rt* agree well with the expectations, so that no indication for a considerable decrease of viability in either race is present (table 1). The numbers, however, are not sufficiently large to exclude minor effects on viability. The average time until hatching of the adult was 70.6 ± 0.83 days for *Rt* animals and 68.7 ± 1.09 days for *rt rt* animals in F_2, and 80.9 ± 1.02 days for *Rt rt* animals and 80.0 ± 1.06 days for *rt rt* animals in the backcross. Neither difference is significant.

Whether the genes *Rt* and *rt* influence the amount of testis pigmentation as well as the quality cannot be decided from the material presented. The testes of *Rt* make on the average a darker impression than *rt rt* testes, but this might be due to greater light absorption of *Rt* pigment as well as to a greater amount.

DEVELOPMENT OF THE TESTIS PIGMENTS

It has been observed that the brown pigment of *Rt* becomes visible somewhat earlier in development than the red *rt rt* pigment. The testis color was observed in a number of larvae, the ages of which were known from the size of the head capsule. Attention was given to animals during the last molt, since this is a short definite stage as compared with the relatively long time spent during the instars. The molting stage is conspicuous by the strict demarcation of the head capsule against the body, due to the withdrawal of the actual head tissue from the head capsule of the preceding instar. It was seen that in the instar before the last already 11 out of 35 *Rt Rt* larvae examined showed traces of pigment in the follicle walls, while none could be observed in 15 *rt rt* larvae. At the time of the last molt, all six *rt rt* testes examined were colorless, whereas they were colored in seven out of 14 *Rt Rt* larvae. During the last instar, 12 out of 17 *rt rt* animals had still colorless testes, whereas all 16 *Rt Rt* animals observed contained more or less pigment in their testes. This observation should however not be used in a discussion of the developmental action of *Rt* and *rt* without further investigation, for it is possible that the same amount of pigment would be visible to the eye in *Rt*, and invisible in *rt rt*, due to the greater light absorption of the former pigment.

TESTS OF ALLELISM OF A AND RT MUTANTS

Since both the *a* series of alleles and the *Rt* and *rt* genes influence the coloration of the testes, the possibility that the *rt* genes are alleles of the *a* series has been investigated. The results of these tests are shown in table 2. Red testes animals (*rt rt*) and heterozygous brown testes animals (*Rt rt*) were crossed to our red-eyed *aa* strain, which has colorless testes. The results obtained are consistent with the hypothesis of allelism as well as with the assumption of independence, if our *aa* strain is homozygous for *rt*. Under the first hypothesis,

we would have to deal with a series of three alleles, a^{Rt} (brown testes), a^{rt} (red testes) and a (colorless testes), with dominance in the order given. Under the assumption of independence, two pairs of alleles would be present, a^+ (colored testes)—a (colorless testes) and *Rt* (brown testes)—*rt* (red testes), the first mentioned gene in each pair being dominant.

The genotypic constitution of the phenotypically brown F_1 from the cross brown testes×red testes would therefore be $a^{Rt}\ a^{rt}$ under the hypothesis of allelism and a^+a^+ *Rt rt* under the hypothesis of independence. The result of the outcross to *aa* should be one brown testes: one red testes in any case, as was actually found (table 2, line 2). The animals with brown testes from this cross should have the genotype $a^{Rt}\ a$ under the assumption of allelism, and a^+a *Rt rt* under the assumption of independence. The males with red testes would have the constitution $a^{rt}\ a$ under the first hypothesis, and a^+a *rt rt* under

TABLE 2

Results of crosses of Ephestia strains with red and brown testes to aa, with tests for goodness of fit for the assumptions of allelism between a and rt, and independence.

TYPE OF MATING	TESTIS COLOR OF OFFSPRING BROWN	RED	COLORLESS (*aa*)	?	TESTS FOR GOODNESS OF FIT OF ASSUMPTION OF ALLELISM			INDEPENDENCE		
					χ^2	n	P	χ^2	n	P
red×*aa*	—	49	—	—						
F_1 (brown×red)×*aa* (f_1)	98	97	—	1	0.003	1	>.9	0.003	1	>.9
f_1 red×f_1 red	—	192	59	—	0.30	2	>.5	0.30	2	>.5
f_1 brown×f_1 red	136	150	76	4	52.62	2	<.001	3.82	2	>.1
f_1×f_1 brown	334	155	144	7	2.32	2	>.3			

the second one. A decision between these two hypotheses was possible by crossing both types of males to their sisters, whose genetic condition could of course not be determined by examination. Two types of females should occur, however, with genetic constitutions corresponding to those of the males. The goodness of fit of the actual numbers obtained is included in table 2.

Under both hypotheses, half the crosses involving red testis males should yield offspring with red testes only, as is indeed the case (table 2, line 3). In the others, a segregation into two brown testes:one red testes:one red eyes (colorless testes) should be obtained, if the testis color genes were alleles of the *a* series; if these genes were independent of the *a* series, a ratio of three brown testes:three red testes:two red eyes should be obtained. In case of linkage between *a* and *rt*, the relative number of animals with red testes should be lower. The result obtained is significantly different from the first assumption ($\chi^2=52.6$, $n=2$, $P<0.001$), whereas it agrees with the second hypothesis ($\chi^2=3.8$, $n=2$, $P>0.1$). The number of animals with red testes is even somewhat greater than expected, so that no indication for linkage between *rt* and *a* is found in these results.

Furthermore, if the father had a brown testis, in half of the cultures all the offspring with black eyes should have brown testes, if the genes for testis color are alleles of the *a* series. The other cultures should yield brown testes, red testes and red eyes in the proportion 2:1:1. Although the latter consequence is supported by the evidence ($\chi^2=2.3$, n=2, P>0.3), no culture among 11 was found which contained animals with brown testes only. That has a probability of occurring by chance of only 0.0005, according to WARWICK'S (1932) tables. This fact, together with the evidence obtained from cultures whose fathers had red testes indicates, that the genes *Rt* and *rt* are independent of the *a* series.

If it is assumed that the *rt* alleles are independent of *a*, a mixture of two kinds of cultures should be obtained from fathers with brown testes, depending on whether the constitution of the mother was a^+a^+ *Rt rt* (nine brown testes: three red testes: four colorless testes) or a^+a^+ *rt rt* (three brown testes: three red testes: two colorless testes). If the assumption of allelism between *rt* and *a* is accepted, they should be homogeneous (two brown testes: one red testes: one colorless testes). Actually, the 11 cultures tested are significantly heterogeneous ($\chi^2=34.1$, n=10, P<0.01). In some of the cultures, it cannot be told to which genotype the mother belonged, although the results from other cultures are significantly different from one possibility but not from the other. Since these latter cultures constitute a minority, the aggregate for all cultures whose fathers had brown testes is given in table 2.

TABLE 3

Influence of different combinations of a^+, a, Rt, and rt on the pigmentation of eyes and testes.

GENOTYPE	TESTIS PIGMENTATION	EYE COLOR
a^+—*Rt*	brown	black
a^+—*rt rt*	red	black
aa—*Rt*	colorless, or slightly brown	red
aa rt rt	colorless, or slightly red	red

These results indicate that the genes *Rt* and *rt* are not alleles of the *a* series. This allows the further conclusion that the seven *aa* animals used for crosses with *rt rt* and with *Rt rt* were homozygous for *rt rt*. The *aa* strain of German origin contained therefore an *rt* gene which had originated independently of the American a^+ strain and had apparently become homozygous.

The interaction of the genes *Rt* and *rt* with a^+ and *a* in the formation of testis pigment is summarized in table 3.

TRANSPLANTATION TESTS

It has been investigated whether or not the qualitative difference between the pigments corresponding to the genes *Rt* and *rt* depends on a qualitative difference in the a^+ substance produced under the influence of these two genes.

Testes were transplanted between larvae of different genotypes before the pigment had begun to form, usually in the larval instar before the last. In these exchanges of testes between the *Rt Rt* and the *rt rt* stock, the grafts were always colored autonomously like the controls (table 4). The animals indicated as questionable in table 4 comprise cases in which the graft was weakly colored or colorless, so that a decision as to the quality of its color was not possible. The autonomous development of the color in the grafts does not prove that no difference exists between the a^+ substances of the two races. For, since the testis is itself a good source of a^+ substance, it could be imagined that each testis forms its pigment with the aid of its own a^+ substance. The decision was possible by implanting testes from our *aa* strain, which, as indicated above, has the constitution *aa rt rt*, and colorless testes, into *Rt Rt* a^+a^+ hosts. Since *aa* testes do not release a^+ substance themselves, but form pigment if grafted into an a^+ host with the aid of the host's a^+ substance, this experiment gives a certain answer to the question. All implanted *aa* testes formed red pigment, indicating that the type of pigment formed depends on the genetic constitution of the graft cell, not on the a^+ substance released by the host.

TABLE 4

Results of testis transplantations between Rt Rt and rt rt animals.

GENETIC CONSTITUTION		PIGMENT OF THE GRAFT		
DONOR	HOST	BROWN	RED	?
Rt Rt a^+a^+	*Rt Rt* a^+a^+	6	—	2
Rt Rt a^+a^+	*rt rt* a^+a^+	12	—	1
rt rt a^+a^+	*Rt Rt* a^+a^+	—	7	5
rt rt a^+a^+	*rt rt* a^+a^+	—	7	2
rt rt a a	*Rt Rt* a^+a^+	—	7	2

DISCUSSION

The genes *Rt* and *rt* affect the quality of the pigment of the testes. Their mode of action is definitely not by means of an influence on the formation of kynurenin, and it would therefore be included in the customary schemes of gene action chains as acting after the formation of kynurenin. It is possible, however, that *Rt rt* acts on some quite different process influencing the formation of the pigment, and might act on this process either early or late.

One more factor is known to be necessary for the formation of eye pigment in Drosophila, another kynurenin-dependent substance. This is the "substrate" of EPHRUSSI and CHEVAIS (1938) which is lacking or changed in *w* Drosophila. EPHRUSSI and CHEVAIS regard this as the actual precursor of the pigment, and ascribe to kynurenin some kind of more auxiliary function. KIKKAWA (1941), on the other hand, considers kynurenin to be the actual chromogen and the substance lacking in *w* Drosophila as an enzyme necessary for the conversion of cn^+ substance into pigment. The first hypothesis seemed originally to be supported by the finding that minute amounts of kynurenin are

apparently sufficient to produce a detectable amount of pigment, as evidenced by the effects of small amounts of a^+ substance in Ephestia transmitted from an a^+a mother to her *aa* offspring, which shows its effects through several larval instars (CASPARI 1936). More quantitative experiments have shown (BEADLE and TATUM 1941) that 1/80,000 mg of kynurenin is already able to induce a detectable amount of pigment in *v bw* Drosophila. Although 1/80,000 mg is a minute amount, it does not seem to be sufficiently small as compared with the total weight of Drosophila (average 0.8 mg) to exclude the participation of kynurenin in the formation of pigment. The alternative hypothesis receives some support from the fact that kynurenin is used up in the process of pigment formation (EPHRUSSI and CHEVAIS 1938; KIKKAWA 1941) and from the hypothesis put forward by BECKER (1939) that the amount of pigment formed may be directly proportionate to the amount of kynurenin present. Difficulties for the assumption of kynurenin as the sole precursor of the pigments depending on it arise from the number of chemically different substances which seem to be derived from it. BECKER was able to distinguish four different groups of substances all dependent on the presence of kynurenin, the skotommins and xanthommins from the eyes of Ephestia, the pigment of the skin of a^+a^+ Ephestia larvae, and the erythrommatin of Drosophila eyes. To this may be added the pigments formed in the testes of Ephestia under the influence of the genes *Rt* and *rt* which both need kynurenin for their development, as evidenced by the fact that both *aa Rt Rt* and *aa rt rt* testes are colorless or weakly colored. These testis pigments show differences in chemical behavior from skotommin, being insoluble in acidified alcohols. This chemical difference between eye and testis pigment in Ephestia would be in agreement with the finding of KÜHN (1936) that the substance released by a^+ brains is able to induce pigment formation in *aa* eyes, but not in *aa* testes, contrary to the a^+ substance released by testes and ovaries, which influences both characters in the same way. Although a number of chemically related substances may possibly arise from the same basic substance under different conditions, the formation of so many different substances from kynurenin is difficult to imagine. Furthermore, the amount of kynurenin formed has hitherto been found to account for only a fraction of the variability of the kynurenin requiring pigments—that is, the difference between the members of the *a* series and perhaps the temperature modifiability in *aa* and a^ka^k strains of Ephestia. Many factors influencing kynurenin-induced pigments are independent of the amount of kynurenin present as, for example, the action of the genes *Rt* and *rt*, the relative rate of development in Ephestia (CASPARI unpubl.), and many of the eye color genes in Drosophila. These facts suggest that the process of formation of these pigments is a complicated one and that kynurenin is at least not the only substance entering into the formation of the pigments.

SUMMARY

A pair of alleles in Ephestia is described influencing the quality of testis pigmentation. The dominant *Rt* causes the formation of a brown pigment, the recessive *rt* of red pigment.

The genes *Rt* and *rt* are not alleles of the *a* series and probably are not linked with this locus.

In transplantation experiments, the pigments corresponding to *Rt* and *rt* are formed autonomously, indicating that these genes do not affect the production of a^+ substance.

The bearing of the results of the probable action of a^+ substance in the formation of pigment is discussed.

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A RE-EXAMINATION OF THE PROBLEM OF MANIFOLD EFFECTS OF GENES IN DROSOPHILA MELANOGASTER

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INTRODUCTION

ALMOST two decades ago, one of us (DOBZHANSKY 1924) found that some mutant strains of *Drosophila melanogaster* differed from each other and from normal flies in certain details of the structure of their genitalia. The mutants concerned were known to affect the eye color (white, ivory), body color (ebony, yellow), eye size (Bar), and bristle shape (forked), but the existence of differences in the genitalic armatures had neither been known nor suspected.

The shape of the spermatheca was chosen as a test character for an examination of the genetic causation of the genitalic differences (DOBZHANSKY 1927). Mutant strains kept in laboratories often differ not only in the mutant genes which produce the obvious external traits for which these mutants are named but also in minor modifying genes which affect various structural and physiological characters of the flies. The observed differences in the shape of the spermatheca might, therefore, be produced either by the same mutant genes which are responsible for the alteration of the eye and body colors, bristle shape, etc., or by independent genes which affect the spermatheca. To discriminate between these possibilities, the mutants were crossed to a wild type strain, the segregation products obtained in F_2 generation were intercrossed again, and the series of crosses were carried for from three to 31 generations. From time to time samples of wild type flies and of flies showing the external characteristics of the mutants were taken, and the shapes of their spermathecae were examined. The differences in the spermatheca shape between the wild type and the mutants ebony, sooty, and tan[2] had not diminished after respectively 31, 16, and three generations of crossing; the differences between the wild type and the mutants cinnabar, white, yellow, and ivory did diminish but did not disappear after the crossing. These results were interpreted to mean that the mutant genes named above affect the spermatheca shape as well as the eye and body colors, but that some independent modifiers of the spermatheca shape are also present.

SCHWAB (1940) has correctly pointed out that the system of crosses used by DOBZHANSKY is not sufficient to exclude the possibility that the apparent effects of the mutant genes on the spermatheca shape are produced by modifiers borne in the same chromosomes as the respective mutants. The system used by SCHWAB himself consisted in crossing the mutants for 40 generations to an especially prepared inbred wild type strain; in this way strains should be obtained which have identical genetic backgrounds except for more or less short chromosome segments carrying the mutant genes under study. Of the eleven

mutants studied by SCHWAB, eight showed significant differences from the wild type in the spermatheca shape before the crossing; however, after the crossing only one of these (dumpy) preserved the difference to a considerable extent, and two (cardinal and wavy) to a slight extent. Of the three mutants which showed no differences in the spermatheca shape before the crossing, one (ivory) has built up a difference after the crossing. These results confirm the existence of modifiers of the spermatheca shape, but leave undecided whether or not the mutant genes known to produce striking visible changes of the eye and body colors, bristle and wing shape, etc., may also act as modifiers of spermatheca shape.

It seems obvious that neither the method of DOBZHANSKY nor the much improved method of SCHWAB is capable of settling the problem. In practice, no matter how long a mutant may be outcrossed to a wild type strain, the possibility will remain that the difference in the spermatheca shape is due not to a pleiotropic effect of the gene responsible for the external characteristics of the mutant but to a modifier closely linked to that gene. The results reported in this paper were obtained with the aid of a quite different method which, we hope, is capable of giving an unambiguous solution of the problem. Instead of attempting to control the modifiers by crossing, we have induced mutations in an inbred wild type strain. The mutations have been detected through the alterations of the externally visible traits. The shapes of the spermathecae in the mutants have then been compared with that in the wild type strain. The probability is great that any difference in the spermatheca shape represents an effect of the same gene which alters the external traits. Although the X-ray treatment with the aid of which we have induced the mutations may cause more than a single mutation in the same fly, these mutations need not be in the same chromosome, much less in the immediate vicinity of each other. Simultaneous mutation of neighboring genes may, of course, take place by chance. If, however, we observe repeated mutations at the same locus, and if simultaneously with the appearance of the external characters of the mutant the shape of the spermatheca undergoes a corresponding change, the probability of the external and the internal changes being due to coincidence becomes negligible.

THE EXPERIMENTAL PROCEDURE

A long inbred Oregon wild type strain described by NEEL (1942) and secured through the courtesy of that author has served as the base in which mutations have been induced. An attached-X yellow stock has been made partly isogenic with the Oregon strain by outcrossing yellow females to Oregon males for four generations. In the fourth generation, Oregon males received 3000 r-units of X-ray; we are indebted to DR. F. M. EXNER who administered the treatment. The male offspring were inspected, and individuals showing mutant characters were isolated. In all, 15,820 males were examined and 144 aberrant individuals found. Most of these were discarded (all the Minutes and "weak" mutants); some were sterile; some were mosaics and failed to transmit their characters; 19 mutants were preserved. Of these, four have white eyes and are designated

white-1, white-2, white-3, and white-4. Alleles of yellow arose ten times; five of them (yellow-1 to yellow-5, tables 1 and 2) have the body as well as the bristles yellow and resemble the generally known allele yellow[1]; the remaining five (yellow-6 to yellow-10) resemble the well known yellow[2] allele in having dark bristles; yellow-9 has a peculiar brownish body color different from that in the previously known alleles. Aside from the whites and the yellows, two ruby, one vermilion, one forked, and one dusky mutants were preserved. The allelism of all these mutants to the previously known ones has been tested in appropriate crosses.

In order to insure the isogeneity of the mutants with the wild type Oregon strain, the mutant males were outcrossed to Oregon females, mutant males obtained in the F_2 generation crossed again to Oregon females from the original strain, recovered in F_4, etc. After repeating this procedure at least thrice for each mutant, homozygous strains were established. The material for measurements of the spermathecae was prepared as follows. Single females heterozygous for a given mutant and for a wild type Oregon chromosome were crossed to mutant males; ten or more such cultures were raised at 24.5°C; daughters homozygous for the mutant and wild type daughters (wild/mutant heterozygotes) were collected and preserved in 70 percent alcohol. The spermatheca shape in the mutant females was compared with that in their wild type heterozygous sibs, the latter serving as controls.

The salivary gland chromosomes of all the mutants were examined for possible chromosomal aberrations associated with the mutants. The white and yellow alleles were examined especially carefully to detect the possible small changes (deficiencies, duplications, or inversions) in the vicinity of the respective loci in the chromosome. None was found. The metaphase chromosomes in the nerve cells of the yellow mutants resembling yellow[1] were examined in order to ascertain that they were X-ray induced mutants rather than yellows resulting from crossing over between the attached X's and the Y chromosomes in the females to which the X-rayed males were mated.

MEASUREMENTS

The spermatheca of *Drosophila melanogaster* is a paired chitinized organ resembling in shape a soldier's steel helmet. The degree of its convexity is described with the aid of the spermatheca index, which is the value obtained by dividing the maximum diameter by the maximum height of the spermatheca. In our material the spermatheca index varies from 1.24 to 1.77 (table 1). The technique of measurements described by DOBZHANSKY (1927) and by SCHWAB (1940) has been followed, except that the flies were preserved in 70 percent alcohol and that a 10 percent, instead of 20 percent, solution of KOH was used for maceration. For each mutant and for its control 100 spermathecae were measured. Of course, only a single spermatheca per fly was measured. It happens that the Oregon strain has about five percent of flies having three spermathecae instead of the normal two, and several flies with four spermathecae have been found. Such individuals were rejected. The spermathecae

TABLE 1

Variation of the spermatheca index in the mutants and the control flies.

	1.25	1.30	1.35	1.40	1.45	1.50	1.55	1.60	1.65	1.70	1.75
white-1	—	7	23	26	25	17	2	—	—	—	—
Control	—	—	3	10	17	26	31	7	6	—	—
white-2	—	2	17	26	33	17	4	1	—	—	—
Control	—	—	—	15	26	30	17	10	1	1	—
white-3	2	7	24	23	30	8	4	2	—	—	—
Control	—	1	7	11	27	23	17	12	1	1	—
white-4	—	1	12	37	39	9	2	—	—	—	—
Control	—	—	—	9	30	29	19	11	2	—	—
yellow-1	1	6	20	36	24	10	3	—	—	—	—
Control	—	—	2	14	30	26	18	10	—	—	—
yellow-2	2	9	22	28	26	7	3	3	—	—	—
Control	—	—	2	16	24	39	16	3	—	—	—
yellow-3	—	10	19	21	25	16	9	—	—	—	—
Control	—	—	2	14	32	29	15	4	3	1	—
yellow-4	—	7	16	36	24	15	1	1	—	—	—
Control	—	1	—	17	27	30	13	8	3	1	—
yellow-5	—	5	23	33	18	14	6	1	—	—	—
Control	—	—	1	8	32	33	18	7	1	—	—
yellow-6	—	—	1	9	27	37	17	7	2	—	—
Control	—	—	1	9	22	27	21	17	2	1	—
yellow-7	—	1	6	16	29	29	17	—	2	—	—
Control	—	—	—	5	19	44	19	10	3	—	—
yellow-8	—	2	8	29	30	17	11	3	—	—	—
Control	—	—	1	4	19	31	24	13	7	1	—
yellow-9	—	1	4	15	36	22	17	5	—	—	—
Control	—	—	1	3	22	39	18	14	3	—	—
yellow-10	—	3	15	37	26	16	2	—	1	—	—
Control	—	—	2	7	24	36	17	6	4	4	—
forked	—	1	10	29	32	20	7	1	—	—	—
Control	—	1	10	24	25	25	12	2	1	—	—
vermilion	1	2	14	39	25	17	1	1	—	—	—
Control	—	—	3	6	26	31	22	7	4	1	—
dusky	—	—	4	10	17	33	22	10	4	—	—
Control	—	—	5	10	33	40	9	3	—	—	—
ruby-1	—	—	4	5	27	38	11	8	5	2	—
Control	—	1	3	14	28	23	22	7	2	—	—
ruby-2	—	—	—	—	11	20	31	24	12	2	—
Control	—	—	1	3	12	28	30	14	11	1	—
Wild type	—	—	—	1	4	20	35	29	9	1	1

were drawn with the aid of a camera lucida (by DOBZHANSKY) and subsequently measured in the drawings (by HOLZ).

The data are summarized in tables 1 and 2. Table 1 shows the observed frequency distributions of the spermatheca index in the mutants and in the corresponding controls. Table 2 presents the mean values with their standard errors, and the ratios (d) of the differences between the mutant and the control

TABLE 2

Mean spermatheca indices in the mutant and the control flies.

	M±m	d		M±m	d
white-1	1.4140±0.0062	10.0	yellow-7	1.4700±0.0064	4.7
Control	1.5085±0.0070		Control	1.5095±0.0055	
white-2	1.4310±0.0059	7.2	yellow-8	1.4485±0.0065	8.0
Control	1.4940±0.0065		Control	1.5225±0.0067	
white-3	1.4110±0.0070	7.4	yellow-9	1.4725±0.0062	4.6
Control	1.4870±0.0076		Control	1.5120±0.0059	
white-4	1.4245±0.0046	9.8	yellow-10	1.4240±0.0059	8.9
Control	1.4995±0.0061		Control	1.5065±0.0072	
yellow-1	1.4090±0.0060	9.1	forked	1.4425±0.0056	1.5
Control	1.4870±0.0062		Control	1.4560±0.0067	
yellow-2	1.4075±0.0071	8.1	vermilion	1.4225±0.0057	9.1
Control	1.4800±0.0054		Control	1.5025±0.0067	
yellow-3	1.4225±0.0072	6.4	dusky	1.5025±0.0290	3.3
Control	1.4850±0.0065		Control	1.4735±0.0053	
yellow-4	1.4155±0.0060	7.9	ruby-1	1.5005±0.0071	1.2
Control	1.4885±0.0070		Control	1.4865±0.0094	
yellow-5	1.4175±0.0066	8.5	ruby-2	1.5560±0.0062	2.1
Control	1.4920±0.0056		Control	1.5370±0.0067	
yellow-6	1.4945±0.0056	1.9	wild-type	1.5615±0.0057	—
Control	1.5110±0.0068				

values and the standard error of the difference. The standard deviations are not shown; they are, of course, in all cases ten times larger than the standard errors. The Oregon strain has a more convex spermatheca than other wild strains of *Drosophila melanogaster:* table 2 shows that the mean index for homozygous wild type flies is 1.5615±0.0057 instead of 1.721±0.005 obtained by DOBZHANSKY (1927) and even higher values found by SCHWAB (1940).

The spermatheca indices in the mutants may now be compared with those

in the controls (table 2). Every one of the four whites has significantly more convex spermathecae (1.414–1.431) than its control (1.487–1.508). Among the ten yellows, only one (yellow-6) does not differ significantly from its control; the single vermilion mutant has a significantly more convex spermatheca than the control. The two ruby mutants do not differ from their controls in the spermatheca shape. Dusky seems to have a flatter spermatheca than its control, but in this case the control value is abnormally low. Very low values are recorded also for forked and for its control.

A careful examination of tables 1 and 2 discloses some interesting details. The wild type controls for the different mutants are decidedly not uniform but vary from 1.456 ± 0.0067 (forked) to 1.5370 ± 0.0067 (ruby-2). Among the whites and the yellows, the lower the value is for the mutant the lower it is for the corresponding control, and *vice versa*. The control flies, it must be remembered, are heterozygous sibs of the respective mutants. The homozygous wild type (Oregon strain) has a relatively very flat spermatheca (1.5615 ± 0.0057). These facts are capable of two interpretations. First, the non-uniformity of the controls may be ascribed to environmental fluctuations which affected differently the different cultures; the flies were raised over a period of several months, and the possibility of environmental changes during this time cannot be excluded. Second, the effects of the mutants on spermatheca shape may be semi-dominant, even though their external effects are more or less completely recessive; hence, the control heterozygotes may have spermathecae intermediate in shape between those in the homozygous mutants and in the homozygous wild type. On this basis, the mutants forked and yellow-6 may be regarded as dominant modifiers of the spermatheca shape (table 2). The second interpretation is seemingly contradicted by the fact that homozygous dusky displays a lower spermatheca index than the homozygous wild type but a higher one than its control (table 2). For this reason, the existence of manifold effects is asserted only for those mutants which show a difference in the spermatheca shape between the homozygotes and the controls.

With some exceptions, repeated mutation at the same locus produces similar constellations of phenotypic effects. The four white homozygotes have similar spermathecae (table 2). But among the ten yellows, homozygous yellow-2 has a distinctly more convex spermatheca than yellow-6 and yellow-9. It seems as though the mutants of the the yellow^1 type have more convex spermathecae than those of the yellow^2 type, but this conclusion is also uncertain on account of the variations among the controls.

DISCUSSION

Every one of the four mutations at the white locus, nine out of the ten mutations at the yellow locus, and the vermilion mutant, all detected because of the changes in the eye or body colors which they produce, have been shown to modify the shape of the spermatheca. No changes of the spermatheca shape are ascertained in the two ruby, in forked, and in the dusky mutants. These negative findings, it must be noted, do not preclude the possibility that the

loci last named may also be concerned with the spermatheca shape. Comparison of a mutant carrying an allele A′ with the ancestral form A is capable of detecting only the effects of the difference A−A′, and not the total effects of either A or of A′. An approximation to a knowledge of total effects of a gene on the development of an organism is attainable solely through comparison of individuals in which this gene is present with such in which the gene is absent. This simple consideration, pointed out for example by DOBZHANSKY (1927), is all too frequently forgotten in discussions of problems of gene action. Furthermore, the manifestation of a mutant gene is subject to modification by the environment as well as by the rest of the genotype of the organism. A lack of a recognizable change in a given environment and in a strain with a given system of modifiers does not preclude the possibility that in other environments and in strains with other genetic residues a change may be observed. Of course, this argument works both ways: it is possible that in other environments and in other strains the mutations at the white, yellow, and vermilion loci would not have produced perceptible changes in the spermatheca shape.

Genes which recognizably alter two or more characters of an organism are said to have manifold effects or to be pleiotropic. The genes white, yellow, and vermilion fall in the category of pleiotropic genes. It is important to analyze the connotations of this teminology. A "character" is obviously an abstraction, though unavoidable for descriptive purposes. Genes produce not characters but physiological states which, through interactions with the physiological states induced by all other genes of the organism and with the environmental influences, cause the development to assume a definite course and the individual to display certain characters at a given stage of the developmental process. Mutations at the yellow locus change the coloration of the head, thorax, and the abdomen; some alleles change the color of the bristles as well, while others do not. But nobody would call the gene yellow pleiotropic for these reasons; we clearly perceive that all the "characters" are reducible to a single change: that of the pigmentation of the body. Mutations at the locus white change the color of the eyes, and some alleles alter also the color of the testicular envelope. Since the relation between the pigmentation of the eye and that of the testis is not altogether clear, one might describe these pigmentations as two separate characters and the gene white as pleiotropic. At present we perceive no relation whatever between the pigmentation of the eyes or of the body on the one hand and that of the spermatheca shape on the other. The fact that some mutant genes alter the pigmentation as well as the spermatheca shape makes these genes distinctly pleiotropic.

This rather naive approach to the problem of the relations between gene and character seems to have an interesting historical background. WEISMANN pictured his "determinants" as discrete particles which are sorted out during the cleavage of a zygote until each of them finds its way into a group of cells or even a single cell in the particular organ of the body the properties of which it is to determine. To WEISMANN, the determinants were representatives in the germ plasm of topographically defined body parts.

The genes are not supposed to act through any such assortment mechanism during cell division, but the notion that genes somehow represent "characters" has affected the thinking of many geneticists. The undeniable fact that repeated mutations of the same locus produce the same single striking change in a certain body part (or a character) seems to lend support to this view; when two or more apparently developmentally unrelated characters are changed, the phenomenon is considered worthy of a special name—pleiotropism or manifold effect.

GRÜNEBERG (1938) has approached the problem in a different way. A gene may influence several characters because it produces several primary effects; this is a "real pleiotropism." Or else, a gene may have only a single primary effect which alters different organs either directly or in succession; this is a "spurious pleiotropism." GRÜNEBERG describes a mutation in the rat which causes thickened ribs, narrowed lumen of the trachea, emphysema of lungs, hypertrophy of the right heart ventricle, blocked nostrils, blunt snout, and other changes resulting in early death. The primary change is here an anomaly of the cartilage which presumably results in the development of the whole syndrome through several causal chains; hence, this case falls into the class of spurious pleiotropism. Rather simple changes in the ontogeny which become ramified later and alter at first sight unrelated characters have also been described by DUNN (1941), LANDAUER (1942) and others. The developmental mechanics of Drosophila being little known, we are unaware of any developmental relations between the pigmentation and the shape of the spermatheca, but it would certainly be rash to deny the existence of such relations.

The difficulty which vitiates GRÜNEBERG's classification of pleiotropisms is that the "primary effects" of no gene are known, and hence it is futile to classify the recorded instances of manifold effects into real and spurious ones. GRÜNEBERG believes that real pleiotropism in his sense does not exist, that the primary action of all genes is always unitary, and that the burden of proof lies on those who deny his views. The problem of gene action, however, can hardly be settled by a fiat. For the time being we can only speculate about what the primary action of genes may be. "Gene action" may consist merely in gene multiplication—that is, in building a copy of itself from the materials available in the cell. The "primary gene products" may be identical in composition with the gene itself but torn loose from the framework of the chromosome. Or they may be, in a sense, waste products, fragments of extra-genic materials left over after the detraction of the components from which a new gene is built (schematically: gene A + substance or substances B taken from the substrate = two genes A + substance or substances C). Since the substances B may be different in different cells of the body, so may be the substances C. Or else, the gene may act as a catalyst in reactions which may be different in different cells, at different periods of the cell cycle, and at different stages of the ontogeny. Self-reproduction of genes may be a process separate from gene action—some geneticists think of genes as "chemical factories" giving out a certain chemical substance or substances.

Experience shows that the best hypothesis is sometimes not the one which in the end proves to describe the facts most truthfully; it may be the one which is most stimulating for devising experiments at a given level of knowledge. It would appear that at the present it is simplest to assume that each gene gives one and only one primary product. For the time being this hypothesis can be neither proved nor disproved. The phenomena of manifold effects have as yet no bearing on it. When some alleles of a gene affect the character A, others B, others C, and still others various combinations of A, B, and C, a multiplicity of the primary gene products suggests itself. However, such a situation is compatible also with the hypothesis of a single primary product of the gene: some alterations of a molecule of a chemical substance may change principally its color, others its boiling point, and still others both "characters" simultaneously.

SUMMARY

Ten mutant alleles of yellow, four of white, two of ruby, and one each of vermilion, dusky, and forked have been induced in a long inbred wild type strain by X-ray treatment. The mutants were detected owing to the modifications of the external characters which they produce. All the whites, nine out of the ten yellows, and the vermilion were found to modify the shape of the spermatheca. The genes white, yellow, and vermilion have manifold effects—they are "pleiotropic" genes. The implications of the concept "pleiotropism" are discussed.

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GENETICS OF NATURAL POPULATIONS. X. DISPERSION RATES IN DROSOPHILA PSEUDOOBSCURA

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INTRODUCTION

THE breeding structure of a population is determined, among other factors, by its effective size (N) and by the migration index (m). The latter constant measures the extent to which the population of a given territory is replaced in each generation by immigrants from the rest of the species or from a territory large enough to have all the genetic variants of a given class at their equilibrium frequencies. The value of the migration index, in turn, is a function of the mobility of the organism. Joint estimates of N and m have been arrived at for certain populations of *Drosophila pseudoobscura*, and some attempt has been made to make separate estimates (DOBZHANSKY and WRIGHT 1941; WRIGHT, DOBZHANSKY, and HOVANITZ 1942). The data reported in the present article were collected in order to test the validity of these estimates.

The experimental work was done in the summers of 1941 and 1942 on Mount San Jacinto, California. This work was made possible by a grant from the CARNEGIE INSTITUTION OF WASHINGTON and by the devoted and conscientious collaboration of MR. BRUCE WALLACE, MRS. N. P. DOBZHANSKY, MISS R. MIRSKY, MR. ALEXANDER SOKOLOFF, MR. H. LEWIS, PROFESSOR C. C. EPLING, and MR. W. HOVANITZ. Acknowledgment is also made to the DR. WALLACE C. AND CLARA A. ABBOTT MEMORIAL FUND of the UNIVERSITY OF CHICAGO for assistance in connection with the calculations.

METHOD

Experiments on the mobility of insects have been made on mosquitoes, house flies, tsetse flies, grasshoppers, codling moths, and other forms. Individuals of the proper species were caught, marked by paint or colored dust, released at a given point, and subsequently recaptured in the neighborhood. With the exception of JACKSON'S (1940) work on tsetse flies, the data give little information on the rules which underlie the dispersal of the species in question. The investigators seem to be most interested in finding out the maximum distance from the point of release at which marked individuals may be encountered. The pioneering work on the dispersal of Drosophila was done by N. W. and E. A. TIMOFEEFF-RESSOVSKY (1940a, b, c). Known numbers of *D. melanogaster* and *D. funebris* marked by easily recognizable mutant characters were liberated at the center of an experimental field. For a week or two after the release of the mutants, baited traps were exposed daily around the point of release, and the

[1] Observational and experimental data by TH. DOBZHANSKY, mathematical analysis by SEWALL WRIGHT.

numbers of the mutant and the wild flies which came to each trap were recorded, whereupon the flies were released again at the point of capture. The distribution of the marked flies on the field gave an idea about the rates of their dispersal; the ratios of the mutant and the wild flies were used to compute the population densities of the wild flies in the territory examined.

An animal actively moving in a uniform two dimensional environment may manifest one of at least three types of dispersal. (1) The movements may be random. The distribution of marked individuals after the lapse of a certain time, for example a day, will be characterized by a variance, σ^2. The mean distance from the point of release at which individuals will be found will be r. The distribution of the marked individuals after two days will have a variance $2\sigma^2$, and the mean distance will be $\sqrt{2}r$. After n days the variance will be $n\sigma^2$, and the mean distance $\sqrt{n}r$. (2) The release of a large number of individuals at a given point raises the population density and may produce a depletion of the food supply and of the shelter space in the immediate vicinity. The flies may tend to escape from the areas of high population density to less densely settled areas. Since the density gradients will become less and less steep as time goes on, the variance of the distribution of the flies will increase at a faster rate at the beginning than toward the end of the experiment. (3) If the flies possess a "homing instinct," each individual will tend to stay within a certain circumscribed territory. JACKSON (1940) found such an attachment to a home territory in tsetse flies. The flies, then, will travel relatively far from the point of release until they establish home ranges and will become largely stationary thereafter. The variance and the mean distance will reach a maximum and tend to become constant.

The experimental fields of the TIMOFEEFF-RESSOVSKYS consisted of traps placed checkerboard fashion at ten meters from each other. Fields of 63 traps (7×9 on the sides) and of 121 traps (11×11 on the sides) were used. Since the marked *D. melanogaster* and *D. funebris* flies barely reach the boundaries of the fields at the end of the experiments, fields of this size are sufficient for the purpose. The experimental procedure had to be modified for our experiments because *D. pseudoobscura* proved to be much more mobile than the species named above. Checkerboards with traps at 20 meters were tried without success; greater distances are impracticable on account of the labor involved. The traps were then arranged in two lines forming a cross, distances between the traps were made ten or 20 meters, and the flies were released at the intersection of the two axes of the cross (fig. 1). Even this arrangement proved satisfactory only for a few days after the release of the marked flies: with the greatest number of traps that could be used the flies reached the ends of the arms of the cross in from two days to a week. As soon as this happened, one of the two axes had to be sacrificed and the other axis extended by adding traps at the ends, thus making a single file of traps. Of course, terrains as uniform as possible were chosen on which to build the experimental fields. Toward the end of the experiments, however, the fields grew to sizes so unwieldy that some non-uniformities were unavoidable.

TECHNIQUE

The third chromosome recessive orange was used as the marking gene in our experiments. It produces a brilliant red eye color which is distinguishable from the wild type by naked eye to even an untrained observer. Orange heterozygotes have repeatedly been found in natural populations, but no homozygotes have ever been met with. It is obviously important to have the released flies equal in vigor to the wild ones. Throughout the experiments F_1 hybrids of two orange strains were used, one of which was extracted from the population of Keen Camp and the other from Andreas Canyon, both localities on Mount San Jacinto, and thus advantage was taken of the hybrid vigor accruing from the crossing. In 1941 the flies were raised on cornmeal-molasses agar and in 1942 on a cornmeal-molasses-oatmeal agarless medium. Care was taken to avoid overpopulation of the culture bottles or any rough treatment that might injure the flies. Probably the best attestation of the fitness of the released flies is the fact that they reproduced in nature after the release: young orange-eyed flies were repeatedly found on or near the old experimental fields in about a month after the release of the parents. The proportion of the marked flies which were recaptured is much higher in our experiments than in those of the TIMOFEEFF-RESSOVSKYS.

Yet, the use of mutant genes in experiments on natural dispersal may be open to doubt; after all, they are raised in laboratory instead of in natural habitats. Experiments in which wild flies captured in the vicinity of the experimental fields were marked by a spot of the "platinum" nail polish and then released in the same way as the orange flies, however, have given concurrent results. The marking is done as follows: a fly held by its wings in a delicate forceps is brushed gently against a needle dipped in "platinum" nail polish. Care is taken to apply the polish only to the middle of the mesonotum between or slightly in front of the dorsocentral bristles. A small speck in this position makes the fly readily recognizable by naked eye, and the insect is unable to brush it off. One must guard against smearing the base of the wing or the space between the thorax and the head. Any improperly marked fly is rejected. Marking hundreds or thousands of flies is arduous work. Since the flies so marked display the same behavior as orange-eyed flies, the latter were used exclusively in the experiments of 1942.

Marked flies were released at the center of the experimental field late in the afternoon, shortly before the hour when wild flies are most active (see below). On the next and the following days baited traps were exposed from the time when the flies become active till dusk when flies disappear. Paper drinking cups with a layer of fermenting banana on the bottom served as "traps." They were inserted in a ring at the top of a wire stand about 20 inches tall, and the stands were placed upright by forcing their lower ends into the soil. The construction of the experimental fields was done with the aid of a string subdivided into ten meter sections. The recording of the flies that have come to the traps was done as follows. The opening of the trap is covered by a glass funnel with a cut off stem. An empty glass vial is applied to the stem, and the flies are made to run into the vial by exploiting their phototropism. The marked and the wild flies

are counted as they walk out of the funnel. When all the flies are in the vial, the latter is closed by a cotton plug and placed at the base of the stand, protected from the sun if necessary. The trap is re-exposed on its stand. Such "revisions" of the traps are repeated at 20 or 30 minute intervals, several observers on different parts of the field working simultaneously. After the last "revision" of the evening, the flies in the vials are released where caught and the traps are taken off. All the traps on the field are revised the same number of times.

ECOLOGICAL DATA

The available information on the ecology of *Drosophila pseudoobscura* will be published elsewhere, only a summary of the most pertinent facts being given here. The flies display a striking diurnal cycle of activity in their natural habitats, a cycle not at all apparent under laboratory conditions. On a warm and cloudless summer day they begin to visit the traps shortly after dawn. The numbers of flies reach a maximum two to three hours after sunrise, and decline thereafter. No flies arrive in the middle of the day. They reappear two to three hours before sunset, reach a new and frequently a very sharp maximum at about sunset, and disappear completely at dusk. This cycle appears to be determined principally by the changes in the light intensity during the day. The times of the morning and evening maxima change through the year in accordance with the changes in the times of sunrise and sunset. Temperature and humidity fluctuations on successive days affect greatly the total numbers of flies in the traps but not the times when they come, except that below 10°C practically no flies appear at any time. On cloudy and rainy days, and on sunny days in the well shaded parts of the forest, some flies remain active at midday. Crevices of tree bark, especially on oaks, serve as shelter for at least some of the flies during the day, and probably also during the night hours. It is very important that flies raised in the laboratory acquire the activity cycle of the wild flies almost at once after the release. For a day or two a few of the laboratory raised flies begin to come to traps about half an hour earlier than the wild ones, but later even this slight difference is no longer observed.

The productivity of traps exposed in different environments is very unlike. In summer the flies are most abundant in forested localities. The traps exposed close to old oak and pine trees are attended very well, those standing in meadows or brushland are less frequented. The food of the adult consists of yeasts and bacteria. Fungus spores are found in the crops of some specimens and in small amounts. The larval food is unknown, but it is most likely the same as that of the adult. Fermenting sap of bleeding trees is one of the probable sources of the food supply. Seasonal cycles differ according to the climatic conditions: at Andreas Canyon (elevation 800 feet) the population density is highest in spring and lowest in midsummer, while at Keen Camp (4300 feet) and Idyllwild (5300 feet) the highest density is reached respectively in June and early July, no flies at all being obtainable in winter. It is now established that at least in California the flies continue to breed throughout the summer, and young flies appear all the time even during the hot and arid periods.

The productivity of a trap is less if many traps are exposed simultaneously

close to each other than if traps are placed far apart. Experiments conducted in the summer of 1942 at Idyllwild, Mount San Jacinto, demonstrated that traps at 20 meters compete with each other, while traps at 40 and 60 meters show no appreciable interference effect. The effective attractive radius of a banana trap, under the conditions of the experiments, can hardly be more than about 30 meters.

THE MAIN EXPERIMENTS

Four experiments made in the summer of 1942 in the vicinity of Idyllwild, California, will be described in some detail; all other experiments of 1941 and 1942 will be dealt with only briefly, although they involve a variety of conditions not represented in the main series.

First experiment

At 5:30 P.M. on June 2, 3051 orange eyed flies not less than two and not more than seven days old were released at the center (trap No. 12, table 1) of a cross-shaped experimental field east of Saunders Meadow. The traps in this

TABLE 1

Numbers of orange and wild flies in the first experiment.

TRAP NO.	EAST-WEST AXIS								
	1	2	3	4	5	6	7	8	9
1	0–2	0–0	0–1	0–1	0–0	0–0	0–0	0–1	0–0
2	0–1	0–5	0–1	0–2	0–0	0–0	0–3	0–1	0–1
3	0–1	2–7	1–3	1–2	0–0	0–0	0–1	0–2	0–0
4	0–2	1–3	0–0	0–0	0–0	1–0	0–0	1–2	0–1
5	0–1	1–6	1–21	3–11	0–1	1–4	1–3	2–3	0–3
6	0–1	0–5	0–1	0–1	1–0	0–0	0–1	0–3	0–1
7	0–1	7–7	1–0	0–1	0–0	2–4	0–3	1–0	0–1
8	4–1	3–10	1–5	6–7	3–2	14–36	4–24	3–9	1–7
9	1–6	13–9	6–18	3–8	2–2	4–5	4–5	5–7	2–6
10	4–2	28–5	7–4	5–0	2–1	11–3	1–0	1–6	0–2
11	6–4	46–12	32–12	6–1	7–0	7–2	2–3	2–2	2–7
12	68–4	84–5	39–3	22–6	18–0	21–7	2–1	3–2	1–5
13	12–1	48–11	24–1	23–6	3–0	7–5	8–2	4–7	2–1
14	3–6	52–17	18–9	10–7	7–0	6–10	5–0	2–0	1–1
15	1–1	3–2	7–0	6–1	2–1	10–4	3–1	0–0	0–1
16	0–4	10–6	8–7	8–4	4–4	7–6	4–0	0–3	3–5
17	1–7	5–17	3–8	4–5	2–6	4–4	0–1	1–8	0–2
18	0–0	3–14	1–8	0–3	1–1	4–10	0–5	1–9	1–5
19	1–5	3–17	5–15	0–5	0–1	1–11	0–1	1–4	0–4
20	0–4	2–11	2–11	1–2	1–4	1–19	1–9	2–11	0–3
21	0–6	3–43	2–23	4–14	0–0	1–33	0–15	1–19	2–17
22	0–7	4–52	4–80	1–29	0–6	3–43	2–29	2–52	0–23
23	0–2	0–8	1–10	0–5	0–0	0–8	0–11	0–7	0–8
24	—	—	0–7	0–1	0–0	0–3	0–0	0–3	0–6
25	—	—	0–4	0–1	0–1	0–3	0–6	0–2	0–1
26	—	—	0–0	0–0	0–0	0–2	0–7	0–11	0–2
Total	101–69	318–278	163–258	103–123	53–30	105–222	37–131	32–174	15–113

TRAP NO.	NORTH-SOUTH AXIS								
	1	2	3	4	5	6	7	8	9
27	0–1	0–14	1–6	0–2	0–0	0–2	0–0	0–2	0–1
28	0–1	0–5	0–5	0–5	0–0	0–0	0–0	1–4	0–1
29	0–3	0–14	0–8	0–6	0–0	0–2	0–0	0–3	0–5
30	0–6	6–37	4–13	0–10	0–3	2–8	0–10	2–13	0–3
31	0–2	1–24	1–16	2–3	2–1	2–8	0–3	3–10	0–14
32	1–1	1–11	6–5	0–3	0–0	1–3	3–0	1–1	0–4
33	1–0	2–19	1–2	0–0	0–0	1–8	0–0	1–5	0–1
34	2–2	7–14	6–7	1–5	0–1	2–4	2–2	1–3	0–1
35	7–2	15–9	8–5	2–3	0–0	4–7	3–4	2–5	1–15
36	17–5	8–8	4–8	6–1	0–0	3–3	1–2	0–2	0–1
37	9–3	20–4	9–5	0–0	1–1	5–2	1–0	0–2	0–2
38	24–?	20–?	18–?	5–?	4–?	5–?	3–0	3–1	0–1
39	32–?	82–?	59–?	18–?	7–?	21–?	11–20	5–23	3–21
40	5–?	9–?	5–?	0–?	0–?	2–?	0–0	3–2	0–3
41	9–?	18–?	15–?	1–?	2–?	19–?	5–17	3–16	4–26
42	0–?	5–?	4–?	1–?	1–?	3–?	2–20	1–27	1–6
43	0–?	2–?	0–?	0–?	0–?	4–?	0–0	0–4	1–2
44	0–?	3–?	10–?	2–?	2–?	1–?	2–12	1–32	0–9
45	1–?	1–?	7–?	2–?	1–?	4–?	5–19	1–18	0–6
46	0–?	4–?	4–?	5–?	3–?	4–?	2–11	1–11	0–10
47	1–?	2–?	4–?	1–?	0–?	2–?	1–16	2–28	0–6
48	1–?	1–?	1–?	0–?	0–?	2–?	0–4	1–19	0–3
49	—	—	0–?	0–?	0–?	1–?	0–7	1–14	0–7
50	—	—	0–?	0–?	0–?	1–?	1–7	1–15	0–10
51	—	—	0–?	0–?	0–?	0–?	0–1	0–3	1–1
Total	110–?	207–?	167–?	46–?	23–?	89–?	42–155	34–263	11–159

and in the following three experiments were spaced 20 meters apart. The east-west axis of the cross consisted of 23 traps; traps No. 1–18 stood in a ravine with a dense growth of *Pinus ponderosa* and *Libocedrus decurrens;* traps No. 19–23 extended onto a flat with large oaks, *Quercus Kellogii*, and onto a meadow. The north-south axis, traps No. 27–37, 12, 38–48, climbed the drier and more sunlit slopes of the ravine with an open stand of *Pinus Coulteri*, *Pinus ponderosa*, *Quercus chrysolepis*, and *Arctostaphylos* sp. The numbers of orange-eyed and of wild flies caught in the traps are shown in table 1. This and the following three tables (tables 1 to 5) are constructed as follows: for each day and for each trap two figures separated by a dash are given; the first of these figures refers to the number of orange-eyed and the second to that of the wild flies recorded. Thus, on the third day of collecting, 32 orange and 12 wild flies were found in trap No. 11 on the east-west axis in the first experiment (32–12, trap No. 11, table 1). No records were made of the numbers of wild flies in traps No. 38–51 on the first six days in the first experiment (the question marks in table 1). On the third day of the first experiment (June 5) the western and the southern arms of the cross were extended by adding three traps to each (No. 24–26 and 49–51, table 1); after nine days so few orange-eyed flies were caught on the field that the experiment was discontinued.

Second experiment

At 6:30 P.M. on June 16, 3297 orange flies two to five days old were liberated at trap No. 16 (table 2) at the center of a cross-shaped field on the slope of a hill west of Saunders Meadow covered with *Pinus Coulteri*, *Quercus chrysolepis*, *Q. Kellogii*, and *Arctostaphylos* sp. The east-west axis consisted originally of traps No. 5–27; on June 18 it was extended by adding four traps to each end (No. 1–4 and 28–31), and on June 19 three more traps, No. 32–34, were added to the west arm (table 2). The north-south axis consisted originally of traps No. 42–52, 16, 53–63; on the second and the third days of the experiment both the north and the south arms were extended by adding traps No. 35–41 and 64–70, respectively (table 2). On the sixth day the east and west arms were sacrificed, and the field became linear from north to south. The point of release was now occupied by trap No. 25, the north arm contained traps No. 1–24, and the south arm No. 26–49. The numbers of orange and wild flies in each trap are shown in table 2.

TABLE 2

Numbers of orange and wild flies in the second experiment. EW—east-west axis; NS—north-south axis. Cross-shaped field for five days, linear on the sixth and seventh days.

TRAP NO.	1 DAY	2 DAYS	3 DAYS	4 DAYS	5 DAYS	6 DAYS	7 DAYS
	EW	EW	EW	EW	EW	NS	NS
1	—	0–15	0–8	0–19	0–20	0–10	0–32
2	—	0–15	0–11	1–21	2–15	0–31	0–71
3	—	0–6	0–9	0–16	0–20	1–20	0–22
4	—	0–8	1–4	1–29	1–21	0–12	0–31
5	2–10	0–9	1–19	2–22	0–36	1–8	0–24
6	2–32	2–20	1–24	2–24	1–22	0–12	0–11
7	3–10	1–6	4–18	1–22	2–33	0–22	0–32
8	0–0	0–6	0–10	0–13	0–23	0–18	0–42
9	2–10	3–16	2–15	3–54	2–30	0–12	0–13
10	0–14	3–16	2–11	2–18	1–7	2–33	0–22
11	5–8	3–14	1–9	1–12	1–21	0–17	0–22
12	4–7	7–12	4–14	2–17	2–25	2–30	1–43
13	0–3	5–33	2–27	5–19	4–40	0–13	2–37
14	18–39	10–47	9–22	6–33	5–35	0–5	0–12
15	33–34	12–38	21–23	10–40	4–24	0–11	1–28
16	128–27	42–55	9–24	16–30	8–49	1–9	1–16
17	15–12	14–15	13–20	8–31	4–17	0–20	1–43
18	11–28	9–25	8–30	8–59	1–10	0–12	2–35
19	2–7	2–8	7–18	3–14	2–9	0–2	2–8
20	3–11	3–29	1–14	6–31	5–44	4–46	2–20
21	5–10	5–9	7–20	4–41	1–18	4–49	8–64
22	1–19	1–8	1–21	3–32	1–9	1–26	1–44
23	3–25	1–11	3–37	7–62	1–51	8–47	5–55
24	2–10	3–18	1–37	5–24	1–11	6–38	2–68
25	1–8	1–2	1–9	7–14	2–28	1–32	6–110
26	1–22	0–38	1–20	1–21	0–13	6–25	1–27

TABLE 2—*Continued*

Numbers of orange and wild flies in the second experiment. EW—east-west axis; NS—north-south axis. Cross-shaped field for five days, linear on the sixth and seventh days.

TRAP NO.	1 DAY	2 DAYS	3 DAYS	4 DAYS	5 DAYS	6 DAYS	7 DAYS
27	6–30	1–25	5–44	0–42	1–22	8–59	11–135
28	—	2–21	2–29	1–22	0–29	4–51	4–43
29	—	4–28	1–15	1–17	0–42	6–14	3–30
30	—	0–12	1–23	2–25	2–15	2–23	3–33
31	—	0–22	1–22	0–24	1–51	3–33	1–40
32	—	—	0–13	0–24	0–26	3–33	0–67
33	—	—	0–12	0–9	0–6	2–57	1–67
34	—	—	0–14	0–14	0–18	1–41	1–67
	NS	NS	NS	NS	NS		
35	—	—	0–29	0–35	0–61	2–36	2–69
36	—	—	0–21	0–23	0–24	2–49	4–122
37	—	—	0–13	1–26	0–9	1–22	4–56
38	—	0–9	0–24	1–24	0–9	1–33	1–89
39	—	2–41	0–24	3–49	0–13	1–62	3–70
40	—	0–46	0–21	0–18	0–16	3–43	3–89
41	—	2–12	0–15	0–9	0–6	1–60	0–54
42	1–9	1–2	0–10	0–11	0–8	0–46	2–101
43	3–21	0–7	1–8	2–8	1–22	0–30	0–50
44	3–14	1–13	1–8	2–14	0–7	0–36	1–99
45	1–7	2–10	0–7	0–17	0–12	1–14	0–123
46	2–6	2–12	1–11	2–25	1–14	0–20	0–49
47	3–5	1–4	1–8	0–17	1–4	0–5	0–19
48	7–16	11–22	3–22	7–40	3–26	0–2	0–41
49	11–25	14–19	19–57	16–60	6–28	0–5	0–35
50	15–35	8–32	7–29	6–38	5–55	0–16	—
51	34–48	26–63	12–33	16–52	5–35	—	—
52	37–37	20–44	18–45	19–79	10–60	—	—
53	37–13	19–16	6–11	4–31	4–16	—	—
54	29–59	20–31	13–21	9–43	14–46	—	—
55	7–30	7–57	7–42	7–61	2–34	—	—
56	8–11	11–18	9–19	15–57	4–36	—	—
57	4–13	7–24	8–27	8–36	4–39	—	—
58	1–6	3–13	3–17	17–44	8–36	—	—
59	1–3	1–10	1–14	2–8	0–32	—	—
60	3–21	7–27	2–25	2–26	4–15	—	—
61	0–19	2–24	1–29	0–18	3–25	—	—
62	0–20	2–19	0–30	2–51	0–24	—	—
63	2–30	2–54	0–38	2–50	0–46	—	—
64	—	0–33	1–32	1–26	0–33	—	—
65	—	3–60	0–29	1–30	1–32	—	—
66	—	1–47	1–66	3–93	1–46	—	—
67	—	3–33	2–48	2–82	2–82	—	—
68	—	—	2–34	0–41	0–54	—	—
69	—	—	0–50	1–38	1–85	—	—
70	—	—	0–62	1–39	2–66	—	—
Total	456–824	312–1389	229–1625	260–2214	137–1996	78–1350	79–2480

Third experiment

At 6:30 P.M. on June 30, 2868 orange flies two to ten days old were released at trap No. 16 (table 3) on a cross-shaped field in Fern Valley. On the first and second days of collecting the east-west axis of the field consisted of traps No. 1–31; the west arm was extended on the third and fourth days by adding traps No. 68–70. The north-south axis included originally the traps No. 32–46, 16, 47–61; on the third and the fourth days both the north and the south arms

TABLE 3

Numbers of orange and wild flies in the third experiment, first to fourth days.

TRAP	EAST-WEST				TRAP	NORTH-SOUTH			
	1 DAY	2 DAYS	3 DAYS	4 DAYS		1 DAY	2 DAYS	3 DAYS	4 DAYS
1	0–26	0–25	0–54	1–28	67	—	—	0–20	0–17
2	0–19	0–24	1–29	0–19	66	—	—	0–17	0–24
3	0–17	1–61	0–50	1–43	65	—	—	0–13	0–13
4	1–21	0–33	0–31	5–30	32	0–19	1–21	0–19	0–14
5	0–12	0–20	0–13	0–20	33	0–10	1–20	1–11	0–9
6	0–16	2–11	1–27	2–28	34	1–17	0–15	0–17	0–11
7	1–28	1–40	5–39	2–24	35	0–16	2–22	1–23	0–22
8	3–111	1–38	9–49	1–45	36	0–23	3–29	2–20	2–22
9	1–53	6–46	5–34	5–36	37	0–21	1–30	2–13	0–17
10	8–42	16–44	4–31	2–18	38	2–11	0–16	0–19	2–10
11	4–9	6–29	2–14	2–6	39	1–12	2–26	2–14	0–7
12	5–4	12–9	5–9	3–6	40	0–21	0–22	1–8	0–9
13	9–7	15–10	7–11	0–5	41	2–15	5–20	1–24	0–15
14	24–8	31–12	10–10	4–11	42	1–4	5–16	5–11	2–7
15	45–21	28–14	10–21	3–14	43	3–14	5–18	0–9	4–10
16	161–30	67–27	12–13	8–19	44	5–7	9–10	2–8	4–6
17	22–6	22–16	13–3	3–19	45	11–5	8–7	1–8	1–5
18	15–8	25–31	11–12	11–19	46	20–8	3–14	5–8	1–17
19	21–26	12–27	7–20	3–27	47	58–16	36–9	14–12	10–7
20	14–16	10–24	11–21	7–18	48	26–14	29–28	20–15	10–10
21	1–12	4–8	4–16	5–55	49	12–10	14–18	8–12	12–17
22	3–8	8–14	4–12	1–11	50	4–10	6–25	8–9	8–23
23	1–9	3–12	3–9	1–16	51	4–6	3–15	4–10	3–11
24	0–3	0–6	1–5	0–5	52	2–16	6–36	7–16	0–12
25	5–60	14–84	16–35	2–25	53	1–10	1–9	6–17	4–5
26	0–30	10–123	11–53	4–41	54	2–10	2–21	2–3	1–11
27	3–48	9–74	11–30	3–26	55	0–12	0–8	1–9	1–7
28	0–39	3–53	2–38	4–42	56	1–41	2–29	2–22	3–26
29	1–10	1–23	0–32	0–9	57	3–90	4–93	5–74	1–57
30	2–22	1–45	1–25	0–26	58	1–60	5–57	3–56	2–34
31	0–8	2–22	1–4	0–11	59	2–49	1–62	0–36	0–33
68	—	—	0–35	0–18	60	1–58	1–26	0–44	1–26
69	—	—	1–16	0–8	61	0–70	0–50	1–26	0–17
70	—	—	1–16	1–8	62	—	—	2–52	1–22
					63	—	—	1–24	1–37
					64	—	—	0–23	0–25
Rotal	350–729	310–1005	169–817	84–736	Total	163–675	155–772	107–722	74–615

were extended by adding traps No. 65–67 and 62–64 (table 3). On the fifth day, July 5, the south and east arms of the cross were sacrificed, and the two other arms were extended. The field now became ⌜-shaped: the northern arm consisted of traps No. 40–16, and the western arm of No. 16, 47–68 (table 4). The experimental field is mostly level and dry, covered with a sparse stand of large *Quercus Kellogii* and *Pinus ponderosa* and a dense undergrowth of the

TABLE 4

Numbers of orange and wild flies on the fifth to seventh days in the third experiment; traps in a ⌜-shaped file, the point of release at No. 16.

TRAP	5 DAYS	6 DAYS	7 DAYS	TRAP	5 DAYS	6 DAYS	7 DAYS	TRAP	5 DAYS	6 DAYS	7 DAYS
40	0–25	0–11	0–30	24	0–12	0–3	0–4	54	0–9	0–4	0–4
39	0–6	0–7	0–20	23	1–18	1–17	0–14	55	2–2	1–6	0–5
38	0–7	0–3	0–33	22	4–10	0–11	0–11	56	1–24	0–17	1–20
37	0–4	0–10	0–14	21	7–14	1–10	1–14	57	2–39	1–33	0–22
36	0–2	0–0	0–4	20	6–7	2–10	2–17	58	1–51	0–13	1–25
35	0–14	0–7	0–12	19	7–41	4–17	2–34	59	0–42	1–33	1–23
34	0–8	0–11	0–7	18	5–19	3–13	1–28	60	0–15	1–20	2–26
33	1–11	0–16	0–27	17	10–20	2–4	1–12	61	1–26	2–20	1–17
32	0–22	0–26	0–12	16	12–47	1–17	4–21	62	0–27	0–24	1–28
31	0–19	0–8	0–6	47	5–19	3–9	1–3	63	1–55	1–26	1–33
30	0–16	0–10	0–7	48	5–23	0–6	0–5	64	0–13	0–5	0–15
29	0–10	0–9	0–8	49	10–8	3–9	1–3	65	0–26	0–15	1–23
28	5–33	4–20	2–45	50	8–32	2–2	0–10	66	0–18	0–23	0–20
27	3–28	0–7	1–24	51	3–5	0–4	1–6	67	0–10	0–12	0–24
26	11–34	9–28	3–29	52	0–7	0–5	1–3	68	0–54	0–33	0–44
25	11–84	14–55	9–123	53	1–3	2–7	0–6	Total	123–1019	58–656	39–921

fern *Pteris aquilina;* the west arm (traps No. 60–68, table 4) debouches into a more densely wooded territory near a stream.

Fourth experiment

Started at 6 P.M. of July 23 by release of 4810 orange flies two to five days old at trap No. 16 of a cross-shaped field south of Idyllwild. Figure 1 shows the numbers of orange-eyed (large straight numerals in the middle) and of wild flies (smaller slanting numerals on the right) recorded in every trap on the first day of collecting, July 24. Figure 1 may be taken as a scheme of all the cross-shaped experimental fields. Since in one day after the release the orange flies had already reached the ends of the east and west arms (fig. 1), from the second day on the field was transformed into a linear one, from east to west. The east arm included the traps No. 55–44, 1–16, and the west arm No. 16, 17–43 (No. 16, of course, being the point of release). The numbers of the orange and wild flies recovered on the second to the fifth days of the experiment are shown in table 5. The central portion of the field is covered with a rather dense growth of young *Pinus ponderosa*, while the peripheral portions are a more open country with oaks, *Quercus Kellogii*.

Examination of tables 2–5 shows that the numbers of wild flies found in different traps vary within wide limits. Day after day some traps attract more flies than other traps. The orange-eyed flies are found at the beginning of the experiments chiefly at or near the point of release, but in the course of time they tend to occur in the same traps which attract most wild flies. This shows

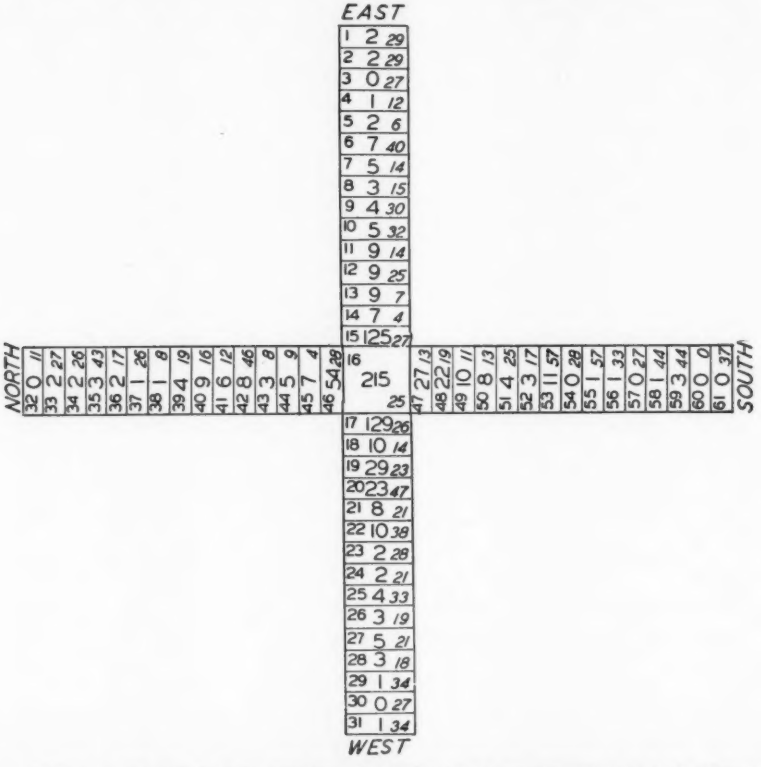

FIGURE 1.—The results of the first day of collecting on the experimental field in the "fourth experiment." The figure at the left in each cell indicates the number of the trap; the figure in the middle, a number of orange-eyed flies; the figure on the right, the number of wild flies.

that the terrains on which our experimental fields were built were not quite uniform. We have pointed out above that traps standing near old trees or in dense vegetation in moister places attract more flies than do traps in more open or drier situations. The flies are attracted to places where they find food and shelter. Fortunately, these non-uniformities were never large enough to produce striking displacements of the flies in one direction from the point of the release of the marked flies.

Another, and more important, non-uniformity in our experiments is intro-

duced by the variation of temperature on different days (table 6). Temperature and humidity records were kept for all the evenings when flies were being collected. The readings were made at about half-hour intervals. As pointed out above, the flies in their natural habitats display two sharp maxima of activity,

TABLE 5

Numbers of orange and wild flies on the second to fifth days in the fourth experiment; traps in a single file, the point of release at No. 16.

TRAP	2 DAYS	3 DAYS	4 DAYS	5 DAYS	TRAP	2 DAYS	3 DAYS	4 DAYS	5 DAYS
55	0–62	0–78	0–109	0–69	17	40–17	11–20	3–18	3–13
54	0–99	0–105	0–183	0–63	18	6–4	2–4	0–9	0–7
53	0–27	0–40	0–203	0–31	19	28–23	8–24	6–27	2–19
52	0–47	0–102	0–174	0–76	20	11–29	5–15	6–24	2–22
51	0–23	0–17	1–31	0–47	21	6–27	3–21	3–14	0–17
50	0–69	0–54	0–69	0–19	22	8–40	2–40	2–39	6–46
49	0–17	0–14	0–24	0–23	23	2–27	0–29	5–33	2–42
48	0–12	0–7	0–9	0–8	24	3–22	2–17	0–10	3–26
47	0–19	0–7	0–12	0–10	25	2–33	2–28	3–33	1–26
46	0–11	0–5	0–13	0–7	26	1–17	2–18	1–15	0–23
45	0–5	0–3	0–13	0–9	27	3–16	3–26	1–27	0–30
44	1–5	0–4	0–25	1–6	28	3–12	1–24	1–31	0–21
1	5–23	1–28	1–29	2–55	29	2–27	2–27	2–23	0–15
2	0–22	1–21	0–33	1–18	30	0–18	2–25	0–33	2–32
3	1–19	2–24	1–20	1–18	31	2–19	1–8	1–15	0–21
4	1–6	1–2	0–10	1–6	32	0–26	0–14	1–23	0–18
5	0–6	0–9	0–6	0–6	33	0–24	0–20	0–19	0–19
6	2–71	3–27	2–27	5–25	34	0–19	0–16	0–13	0–17
7	4–11	1–9	4–13	0–8	35	0–30	0–19	0–22	0–15
8	1–15	3–8	1–10	2–9	36	1–14	0–23	1–16	0–26
9	4–27	0–26	0–23	2–21	37	0–19	0–19	0–14	0–19
10	8–29	4–29	1–11	1–13	38	0–45	0–34	0–30	0–55
11	11–21	3–26	2–8	2–28	39	0–38	0–39	2–19	0–11
12	19–32	7–23	4–15	0–18	40	0–22	0–13	0–8	0–15
13	14–16	2–17	0–5	4–13	41	0–60	1–44	1–25	1–38
14	2–9	2–8	0–9	1–5	42	0–22	0–10	0–17	0–16
15	45–34	10–33	6–22	4–30	43	0–47	0–50	0–40	0–24
16	70–43	15–15	11–24	2–22	Total	306–1477	102–1368	73–1727	51–1296

one a short time before sunset and the other in a few hours after sunrise. We believe that the temperature at the time of these maxima of activity is most important as far as the fly movements are concerned. The temperatures at the times of the evening maxima (F°) are shown in table 6. We have only a few records of the temperatures during the morning maxima of activity on the same days. However, the summer climate at Idyllwild is remarkably equable, and the morning and the evening temperatures on the same day are in general closely correlated.

THE DISTRIBUTION OF THE WILD FLIES

In making a detailed analysis of the data it will be well to consider first the

implications relating to the wild flies. These were distributed sufficiently uniformly throughout the regions studied that some were caught in nearly all the traps on each day. In the first experiment in which the average number caught per trap was small (one to 13 on different days) at least one wild fly was caught in 309 of the 369 traps (84 percent). In the other experiments, in which the average caught per trap was higher on all days (14 to 52), there were only three among 1072 traps which failed to yield at least one wild fly.

On the other hand, the distributions were obviously not random. In a random distribution, the numbers on a given day should exhibit a Poisson distribution with variance approximately equal to the mean. But, as brought out in table 6, the variance of numbers per trap was always much greater than the mean ($\sigma^2/m = 9.7$ in experiment I, 8.6 in experiment II (first five days)) 14.1 in experiment III (first four days) and 5.4 in experiment IV (single line, five days).

TABLE 6

Statistics of wild flies caught on successive days in experiments I to IV. The first column gives the day, and the second column the temperature (F), the third column the number (n) of flies caught in those traps in which counts were made on the first day, the fourth column gives the mean number per trap (m), the fifth column the standard deviation of the numbers (σ), and the sixth column the ratio, σ^2/m as an index of randomness of dispersion ($\sigma^2/m = 1$ if Poisson distribution). The figures in experiment I are based on 34 traps, spaced 20 m apart in three arms of a cross. In experiments II, III, and IV they are based on 45, 61, and 61 traps respectively, similarly spaced, in four arms of a cross. Similar data (n', m', σ' and σ'^2/m') are given in additional columns relating to two arms of the cross in which trapping was continued for a greater number of days in experiments II, III, and IV (23, 31, and 31 traps respectively).

		I N.E.W.							
DAY	TEMP.	n	m	σ	σ^2/m				
1	56°	95	2.8	2.1	1.6				
2	67°	437	12.9	11.9	11.0				
3	66°	327	9.6	14.1	20.5				
4	59°	159	4.7	5.5	6.4				
5	55°	35	1.0	1.7	2.7				
6	65°	261	7.7	10.3	13.7				
7	62°	139	4.1	6.8	11.2				
8	63°	208	6.1	9.1	13.6				
9	60°	151	4.4	5.4	6.5				
Total		1812	5.9	7.4	9.7				
		II N.E.S.W.				II′ N.S.			
DAY	TEMP	n	m	σ	σ^2/m	n'	m'	σ'	σ'^2/m'
1	70°	824	18.3	12.9	9.0	475	20.7	14.3	9.9
2	71°	981	21.8	15.6	11.1	576	25.0	17.9	12.8
3	70°	997	22.2	11.6	6.0	535	23.3	13.5	7.8
4	71°	1461	32.5	17.4	9.4	816	35.5	19.4	10.6
5	68°	1197	26.6	14.1	7.5	669	29.1	15.7	8.5
6	73°	—	—	—	—	718	31.2	17.1	9.4
7	63°	—	—	—	—	1203	52.3	33.8	21.8
Total		5460	24.3	14.3	8.6	4992	31.0	18.8	11.5

		III N.E.S.W.				III′ N.W.			
DAY	TEMP.	n	m	σ	σ^2/m	n′	m′	σ'	σ'^2/m'
1	70°	1404	23.0	21.6	20.3	538	17.4	13.1	9.8
2	72°	1777	29.1	22.4	17.2	879	28.2	24.8	21.9
3	77°	1323	21.7	15.3	10.8	540	17.4	11.2	7.2
4	71°	1179	19.3	12.4	8.0	550	17.7	11.6	7.6
5	74°	—	—	—	—	717	23.1	17.5	13.2
6	75°	—	—	—	—	427	13.8	11.2	9.2
7	74°	—	—	—	—	575	18.5	22.1	26.4
Total		5683	23.3	17.9	14.1	4222	19.5	15.9	13.6

		IV N.E.S.W.				IV′ E.W.			
DAY	TEMP.	n	m	σ	σ^2/m	n′	m′	σ'	σ'^2/m'
1	71°	1465	24.0	12.8	6.8	740	23.9	10.1	4.3
2	72°	—	—	—	—	715	23.1	13.1	7.4
3	74°	—	—	—	—	631	20.4	9.1	4.0
4	78°	—	—	—	—	616	19.9	9.6	4.7
5	69°	—	—	—	—	655	21.1	11.7	6.5
Total		1465	24.0	12.8	6.8	3357	21.7	10.7	5.4

That the departures from randomness were due to persistent local conditions is shown by the correlation between the numbers caught in the traps on the first day and on subsequent days (table 7). The average correlation between first and second day was .645 based on 171 traps. There was only a slight falling off in the correlation between first and third day (.616), and first and fourth day (.598) based on the same 171 trap stations on each day. That between first and fifth day (.488) is based on the same 110 trap stations in experiments I, II, and IV but does not include experiment III. The average correlation between first day and sixth to ninth days (a total of 136 trap stations in experiment I only) was appreciably lower (.381). On taking into account the rate of

TABLE 7

The correlation between the numbers of wild flies caught in traps on the first day and the numbers caught in the same traps on subsequent days. The number of traps is indicated in parentheses under the designation of the experiment (I to IV). The standard errors would be somewhat larger than indicated by the formula $SE_r=(1-r^2)\sqrt{n}$ *because of correlation between adjacent traps. The unweighted averages are given in the last column.*

DAYS	I (34)	II (45)	III (61)	IV (31)	AV.
1–2	.552	.699	.633	.693	.645
1–3	.547	.540	.740	.560	.616
1–4	.532	.542	.647	.657	.598
1–5	.565	.417	—	.507	.488
1–6	.379	—	—		
1–7	.311	—	—		.381
1–8	.455	—	—		
1–9	.380	—	—		

dispersion of the flies, discussed later, it becomes apparent that these correlations must depend on more or less persistent local conditions. The tendency to aggregate about old oak and pine trees appears to be the major factor here.

ACTIVITY AND TEMPERATURE

In experiment I (table 6), the average number of wild flies caught per trap varied rather closely with the temperature which was lower throughout this experiment (55° to 67°) than on any day of any other experiment with one exception (63° on the seventh day of experiment II). The average number caught per trap in experiment I was much lower than in the other experiments, but this might be attributed to earliness of season as well as to an effect of low temperature. However, there can be little doubt that the exceptionally low catches on the first and fifth days at temperatures of 56° and 55° respectively reflect low activity of the flies at these temperatures. As will be brought out later, the catches of orange flies and the amounts of dispersion corroborate this conclusion.

On the other hand, there is no relation, that can be relied upon, between temperature and catch per trap in experiments II, III, and IV. It happens that the largest average per trap (52.3 on the seventh day of experiment II) came at the *lowest* temperature (63°) in these experiments. This might suggest an optimum at about 63°. The average catch for these three experiments was so nearly the same that they may be thrown together without serious error. The correlation between mean number per trap and temperature is $-.64$, based on 19 entries (m table 6 where available, otherwise m′). The regression, of catch on temperature is $-1.57 \pm .45$ flies per degree Fahrenheit or 3.5 times the standard error. These values, however, are greatly affected by the record on the one exceptional day referred to above. Omitting this, the remaining 18 days yield a correlation of only $-.28$ and a regression of $-.49 \pm .42$.

THE FORM OF DISTRIBUTION OF THE RELEASED FLIES

The first question in connection with the orange flies concerns the relation between observed distribution along a single line of traps, or along two lines forming a cross, and the total actual distribution in the area. It is obvious from inspection of tables 1 to 5 that most of the flies were still close to the point of release after a day and that they were moving out rapidly in all directions on subsequent days. This scattering, however, is not wholly at random. The numbers in the various directions (table 8) differ much more than expected purely from chance, ($\chi^2 = 630$ $n = 66$) but the evidence that the wild flies tend to cluster at certain points makes a purely random scattering unlikely *a priori*. There was in fact an excess of orange flies in the same directions in which wild flies were caught in excess. Table 8 shows the numbers of wild flies and of orange flies captured on each day at the point of release (c) and in each direction from this. In the case of the wild flies, only those points are considered in which traps were put out on the first day. All traps are considered in the case of the orange flies. The two populations are not strictly comparable in any case because of the changing distributions of the orange flies, in comparison with the relative stability of the wild population. The 2×2 correlation tables

TABLE 8

The numbers of wild flies and of the released orange flies caught at the central trap (C) and in north (N), east (E), south (S), and west (W) lines of traps on successive days. In the case of the wild flies, the same 11 trap stations in each direction are considered on each day of experiments I and II, the same 15 traps in each direction in experiments III and IV. In the case of the orange flies all traps are included. In experiment I, wild flies were not recorded in the south arm of the cross.

		WILD						ORANGE					
	DAY	C	N	E	S	W	TOTAL	C	N	E	S	W	TOTAL
I	1	4	26	22	—	43	95	68	37	15	73	18	211
	2	5	159	69	—	204	437	84	60	101	147	133	525
	3	3	80	66	—	178	327	39	40	49	127	75	330
	4	6	38	34	—	81	159	22	11	24	35	57	149
	5	0	6	6	—	23	35	18	3	15	20	20	76
	6	7	47	54	—	153	261	21	20	40	69	44	194
	7	1	21	43	—	74	139	2	10	12	32	23	79
	8	2	50	36	—	120	208	3	11	15	23	14	66
	9	5	47	29	—	70	151	1	1	5	10	9	26
II	1	27	223	167	225	182	824	128	117	69	92	50	456
	2	55	228	217	293	188	981	42	90	46	88	46	312
	3	24	238	192	273	270	997	9	63	48	56	53	229
	4	30	361	274	425	371	1461	16	75	36	77	56	260
	5	49	271	296	349	232	1197	8	32	25	50	22	137
	6	32	265	—	421	—	718	1	30	—	47	—	78
	7	110	393	—	700	—	1203	6	28	—	45	—	79
III	1	30	203	394	472	305	1404	161	46	101	117	88	513
	2	27	286	416	486	562	1777	67	45	119	110	124	465
	3	13	212	422	361	315	1323	12	23	59	84	98	276
	4	19	181	333	296	350	1179	8	16	31	58	45	158
	5	47	365	—	—	305	717	12	71	—	—	40	123
	6	17	222	—	—	188	427	1	40	—	—	17	58
	7	21	376	—	—	178	575	4	22	—	—	13	39
IV	1	25	300	311	425	404	1465	215	107	190	91	230	833
	2	43	—	341	—	331	715	70	—	118	—	118	306
	3	15	—	290	—	326	631	15	—	40	—	47	102
	4	24	—	241	—	351	616	11	—	23	—	39	73
	5	22	—	273	—	360	655	2	—	27	—	22	51

of excess and deficiency in the number of wild and orange flies in the various directions on each day are shown below. A few cases in which the number in a direction was the exact average are omitted. On certain days only two directions are available for comparison, on others three or four directions. They are given separately.

		orange +	orange −
wild	+	7	1
	−	7	7

2 directions

		orange +	orange −
wild	+	7	2
	−	4	8

3 directions

		orange +	orange −
wild	+	18	7
	−	5	18

4 directions

Since there is only one degree of freedom for each pair of entries in the cases in which counts were made in only two directions, two degrees of freedom for each set of three entries for days with counts in three directions and three degrees of freedom from the sets of four entries for days with counts in four directions, the frequencies above were multiplied by $\frac{1}{2}$, $\frac{2}{3}$, and $\frac{3}{4}$, respectively. The value of χ^2 for the combined table, weighted in this way is 13.7, which, with one degree of freedom, is highly significant.

There was very little wind during the experiments, making it unlikely that there was any systematic drift of the released flies from this cause. It is probable (as pointed out) that differences in terrain were in part responsible for the differences in the numbers caught in different directions. In the main, however, it appears from the tables that local differences in conditions are responsible. We will accordingly treat the distributions as centered at the point of release rather than at the intersections of the east-west and north-south means.

The simplest hypothesis with respect to the dispersion is that the distribution on any day is a radially symmetrical bivariate normal one except as modified by local conditions. In this case, the distribution along a line through the center should have the same form and variance as that along any line whether through the center or not and hence should be representative of the total in a given direction. Unfortunately it is obvious from inspection that the observed distributions are far from normal, at least on the first day or two. There are excessive frequencies at the point of release, associated with excessive scattering of other flies—that is, distributions that are leptokurtic instead of normal. In a normal frequency distribution the fourth moment about the mean is three times the square of the second moment about the mean (and less than three

TABLE 9

The kurtosis of the distribution of orange flies along a line of traps, as measured by $n\Sigma r^4 f/(\Sigma r^2 f)^2$. *The figures under I, II, III, and IV are based on four arms of the cross, while the figures under II′, III′, and IV′ are based on the two arms that were continued to the ends of the experiments. The last column gives the weighted average of I, II, III, and IV. II′, III′, and IV′ were used where II, III, and IV were not available.*

DAY	I	II	II′	III	III′	IV	IV′	AV.
1	9.82	7.64	7.07	10.41	10.25	8.34	8.44	8.88
2	5.69	5.02	4.93	4.41	3.57	—	5.86	5.21
3	4.24	4.39	5.28	2.76	2.32	—	4.15	3.84
4	4.30	3.61	4.01	3.04	3.03	—	3.97	3.68
5	4.45	4.03	4.62	—	2.66	—	3.01	3.59
6	3.47	—	3.55	—	1.77	—	—	3.21
7	3.05	—	2.65	—	1.93	—	—	
8	2.43	—	—	—	—	—	—	2.73
9	3.89	—	—	—	—	—	—	

times if the moments are taken about any other origin). Table 9 shows the value of $n\Sigma r^4 f/(\Sigma r^2 f)^2$ on all days of the four experiments, r being the distance from the point of release (in 20 meter units) and f the corresponding frequency.

The figures under I, II, III, and IV are based on the cross shaped plots with the frequency in the central trap doubled, as an element in both the east-west and the north-south distribution. The figures under II′, III′, and IV′ are based on the two lines of traps that were continued to the ends of those experiments. The

TABLE 10

Estimates of the number of orange flies, that would have been caught on successive days of each experiment if traps had been placed throughout the region of dispersal in a grid with 20 meter spacing, assuming no competitive effect. Without this assumption, all estimates must be multiplied by an unknown constant, K. Column (1) gives the experiment and day, column (2) the ratio, R, of wild flies caught on the day in question in the traps involved in table 6 to that on the first day (second day in experiment I) to be used as an index of activity, column (3) gives an estimate of orange flies by the formula $2\pi\Sigma rf$ which gives no weight to flies caught at the point of release and is undoubtedly an underestimate. In column (4) this is divided by the index, R, of column (2), column (5) gives an estimate by the formula $2\pi\Sigma rf+c$ which is probably a slight overestimate; in column (6) this is divided by the index, R, of column (2). The last column gives the ratio of the estimates with (5) and without (3) inclusion of the central station. These are based on all four arms of the cross (except that only three arms were available for calculation of the ratio, R, in experiment I).

	(1) DAY	(2) R	(3)	(4) (3)/(2)	(5) (3)+c	(6) (5)/(2)	(7) (5)/(3)
I	1	.217	498	(2295)	566	(2601)	1.136
	2	1.000	1830	1830	1914	1914	1.046
	3	.748	1481	1980	1520	2032	1.026
	4	.364	655	1799	677	1860	1.034
	5	.080	303	(3787)	321	(4012)	1.059
	6	.597	1067	1787	1088	1822	1.020
	7	.318	468	1472	470	1478	1.004
	8	.476	496	1042	499	1048	1.006
	9	.346	160	462	161	465	1.006
II	1	1.000	1516	1516	1644	1644	1.084
	2	1.191	1695	1423	1737	1458	1.025
	3	1.210	1379	1140	1388	1147	1.006
	4	1.773	1904	1074	1920	1083	1.008
	5	1.453	1013	697	1021	703	1.009
III	1	1.000	1598	1598	1759	1759	1.101
	2	1.266	2630	2077	2697	2130	1.026
	3	.942	2303	2446	2315	2458	1.005
	4	.842	1291	1537	1299	1546	1.006
IV	1	1.000	2975	2975	3190	3190	1.072

standard error in the case of a normal distribution is approximately $\sqrt{24/n}$ where n is the total number of independent entries. The entries cannot be considered to be wholly independent here because of the tendency of the flies to cluster in particular localities. Nevertheless, there can be no doubt that the departures from three are highly significant for several days. Leptokurtic distributions of this sort would result from the superposition of two normal distributions, one of small standard deviation relating to flies that move only short

distances if at all and one of large standard deviation relating to flies that make at least one long flight. In the course of time it is likely that all surviving flies will fall in the latter category, giving a progressive approach toward normality such as is observed.

THE DECLINE IN NUMBERS IN THE RELEASED POPULATION

In order to make approximate estimates of the properties of the total distribution, it will be assumed that the latter may be represented sufficiently well for this purpose by a radially symmetrical figure in which the ordinate (z) at a given distance (r) in any direction from the center (point of release) is proportional to the average ($\bar{f}$) of the frequencies at this distance in the four (or two) observed directions. The total frequency in the bivariate distribution can be represented by $\int_0^{2\pi}\int_0^{\infty} rzdrd\theta$, θ being the angle between the direction of the fly from the center and a given axis, and $rdrd\theta$ the element of area. The total number that would have been caught if traps had been placed at all intersections in a grid of lines spaced at 20 m intervals both ways instead of at 20 m intervals along single lines may thus be estimated roughly as $2\pi\Sigma r\bar{f}$, assuming no competition between traps.

It is probable, however, that there is some competition between traps as close as 20 meters. DOBZHANSKY and EPLING (in press) concluded from carefully controlled tests that traps in a 20 m$\times$20 m grid catch only about 50 percent to 70 per cent as many flies as traps in a 40 m$\times$40 m or more widely spaced grid. The estimate of the potential catch in a grid with 20 m$\times$20 m spacing may then be represented by $2\pi K\Sigma r\bar{f}$ where K is an unknown constant, probably between .50 and one, designed to measure the competitive effect in a grid as compared with a single line.

This formula makes no allowance for the catch at the point of release, especially important on the first day. It does not appear to be possible to make accurate allowance for this, since the catch per trap cannot be considered as strictly proportional either to the ordinate at the appropriate distance or to the frequency in the 20 m$\times$20 m square centered at this point in the model bivariate distribution. It is probable, however, that the frequency in the central trap (c) should be given something approaching full weight, giving as an upper limit for the total catch in a 20 m$\times$20 m grid, $2\pi\Sigma r\bar{f}+c$ if $K=1$.

Table 10 gives both estimates (omitting factor K), ($2\pi\Sigma r\bar{f}$) in column 3 and ($2\pi\Sigma r\bar{f}+c$) in column 5. The ratio (5)/(3) is given in column 7. Table 11 gives similar estimates for the single lines that were continued to the ends of the experiments. In no case do the two estimates differ to an important extent except on the first day in which that in which c is added is on the average 10 percent the larger. We shall use this larger estimate in later discussions.

In the case of the orange flies there are four reasons for changes in the actual numbers caught from day to day: (1) a systematic tendency to decrease because of the dispersion from the center with consequent increase in the average distance from the lines of traps, (2) real changes in the size of population (necessarily decreases since no orange flies were added), (3) changes in the proportion caught due to differences in activity, (4) accidents of sampling. The for-

mulae discussed above are intended to obviate the first of these. It may be seen by inspection that their application gives population estimates which do not fall off at the rapid rate found (with a few exceptions) in the actual captures. Indeed in experiments I, II, and III the estimates for the first day are less than those for certain later days by amounts too great to be attributed to

TABLE 11

Similar estimates to those of table 10 but based on the two arms of the cross in which trapping was continued to the ends of the experiments.

	(1)	(2) R	(3)	(4) (3)(2)	(5) (3)+c	(6) (5)/(2)	(7) (5)(3)
II	1	1.000	1772	1772	1900	1900	1.072
	2	1.213	2240	1847	2282	1881	1.018
	3	1.126	1423	1264	1432	1272	1.006
	4	1.718	2331	1357	2347	1366	1.007
	5	1.408	1219	866	1227	871	1.006
	6	1.512	1451	960	1452	960	1.000
	7	2.533	1561	616	1567	619	1.005
III	1	1.000	1398	1398	1559	1559	1.115
	2	1.626	2790	1716	2857	1757	1.024
	3	1.004	2422	2412	2434	2424	1.005
	4	1.022	1106	1082	1114	1090	1.007
	5	1.333	2020	1515	2032	1524	1.006
	6	.794	1357	1709	1358	1710	1.001
	7	1.069	983	920	987	923	1.003
IV	1	1.000	3704	3704	3919	3919	1.058
	2	.953	2975	3122	3045	3195	1.023
	3	.841	1558	1853	1573	1870	1.009
	4	.821	1470	1790	1481	1804	1.008
	5	.873	1106	1267	1108	1269	1.002

accidents of sampling. There were clearly considerable differences in the proportions caught on different days.

In the case of the wild flies the first of the above causes of change does not hold, but the real population changes may be in either direction. There is no obvious trend in experiments I, III, and IV, but there is a suggestion of a rising trend in experiment II. There are also irregularities from day to day that indicate changes in the proportions caught.

The almost perfect correlation between the catch of wild flies and temperature in experiment I has been noted. The estimates for the orange flies show closely similar fluctuations. It seems reasonably certain, therefore, that the estimates for the orange flies are low on certain days, especially on the first, fourth, and fifth days of experiment I, because of exceptionally low temperatures on those days (56°, 59° and 55°, respectively). On the other hand, little significance can be attributed to differences in temperatures above this in view of the relations in the wild flies discussed above. Excluding these three days in

experiment I, the estimate for the orange flies (column 5, tables 10 and 11) indicate on the whole a downward trend. In table 12 these estimates are given on a scale on which 100 is that for the first day (second day in experiment I). Those relating to days on which the temperature was below 60° are in parentheses and are omitted in calculating the average. The figures used are those for captures in four directions where available, but two directions where not.

TABLE 12

Estimates of the relative numbers of orange flies on each day of each experiment, taking the number on the first day as 100 (second day in experiment I). These are based on the entries in column 5, table 10, as far as possible, on column 5, table 11, for the later days of experiments II, III, and IV. These are independent of the counts of wild flies. Estimates for three days in experiment I in which the temperature was below 60° F (in parentheses) were not used in calculating the unweighted average given in the last column.

DAY	I	II	III	IV	AV.
1	(30)	100	100	100	100
2	100	106	153	78	109
3	79	84	132	40	84
4	(35)	117	74	38	76
5	(17)	62	130	28	74
6	57	76	87	—	73
7	25	82	63	—	57
8	26	—	—	—	—
9	8	—	—	—	—

The regression of relative number on day is found to be 7.5 ± 1.4 for the averages from the first to seventh day (at least three experiments). This is 5.4 times the standard error which with five degrees of freedom may be considered significant. (Probability about .003 of being exceeded by accidents of sampling.) The ratio of this regression to the estimate on the middle (fourth) day indicates a rate of decrease of about 9.2 percent per day in the orange population.

Another method of attack on the problem is to use the ratio of wild per station on a given day to that on the first day as an index of relative activity by which the estimate of the number of orange flies on the same day may be divided in order to reduce the latter to comparability with the number on the first day. This is done in columns 4 and 6 of tables 10 and 11. The entries in column (6) table 10, where available, table 11 otherwise, are transformed in table 13 to a scale on which the entry for the first day becomes 100. The resulting numbers fall off more or less regularly from day to day in experiments IV and II. In experiment I the records of the first and fourth days are brought fairly well into line with the others, but there is overcorrection to an extreme extent of the figure for the fifth day. However, the catch of wild flies (35) was too small on this day to give an adequate basis for calculations. In experiment III the results are somewhat less irregular in table 13 than in table 12 but there is a much less rapid decline than in the other experiments. The unweighted averages for the first seven days, omitting nothing, yield a regression of 6.4

± 1.7. This is 3.7 times its standard error and probably significant. Taken in ratio to the value on the fourth day, it indicates a rate of decrease of population of about 8.2 percent per day instead of 9.2 percent from the actual numbers (temperatures of 60° or more).

Neither of these methods of estimating the rate of decline in the numbers of the released flies is unequivocal. Those of table 12 are entirely independent of the wild flies, but may merely reflect an average decrease in activity of the

TABLE 13

Estimates of the relative numbers of orange flies in each day of each experiment, as in table 12 but based on the entries in column 6 in tables 10 and 11 and thus supposedly corrected for differential activity on different days by use of the index (R), based on the numbers of wild flies caught. All figures are used in the unweighted average, including the highly unreliable estimate for the fifth day of experiment I. Without this the average for the fifth day becomes 58.

DAY	I	II	III	IV	AV.
1	100	100	100	100	100
2	74	89	121	82	91
3	78	70	140	48	84
4	72	66	88	46	68
5	154	43	98	32	82
6	70	51	110	—	77
7	57	33	59	—	50
8	40	—	—	—	—
9	18	—	—	—	—

flies during the four experiments. Those of table 13 should be free on the average from the effects of external conditions but may merely reflect (in reverse) an average rising trend in the wild populations of the four experiments. However, there is no apparent reason for a systematic deterioration of external conditions in all experiments nor for a systematic rising trend in the four wild populations, recalling that they were started at intervals from June 2 to July 23. The fact that both methods give approximately the same rate of decline tends to indicate that this rate, 8 or 9 percent per day, is approximately the actual rate of decline in the numbers of the released flies. The estimates for the eighth and ninth days of experiment I were not used in either case, but as far as they go indicate an even more rapid decline in numbers after seven days.

THE DENSITY OF THE WILD POPULATIONS

Another question on which estimates are of interest is the density of the wild population. We have estimated that 566K orange flies would have been caught on the first day of experiment I (column 5, table 10) if traps had been set in a grid with 20 m$\times$20 m spacing, extending sufficiently to cover the entire range. But 3051 flies had been released. Assuming a 9.2 percent loss per day, the number of orange flies on hand after one day should be 2770. The proportion which would have been captured under the postulated conditions is .204K($=566K/2770$). On this day 95 wild flies were caught in 34 traps. The number that would have been caught per trap in a grid with 20 m$\times$20 m spac-

ing may be estimated as $2.79K(=95K/34)$, assuming the same factor K as in the case of the orange flies to allow for the greater competition between traps in a grid than in single lines. The actual number of wild flies per 400 square meters may thus be estimated at $13.7(=2.79K/.204K)$ or 3.4 per 100 square meters.

The catch on this first day was poor, presumably because of the low temperature (56°). It is therefore desirable to make an estimate from the second day's catch which was the largest in this experiment. It will be assumed that the original 3051 orange flies have been reduced to $2515(=3051\times.908^2)$. The proportion of those which would be caught in a grid with 20 m × 20 m spacing is estimated at $.761K(=1914K/2515)$. On this day 437 wild flies were caught in 34 traps or 12.85 per trap. The estimated density of the wild population on the same basis as for the first day is 4.2 per 100 square meters. This is probably somewhat more accurate than the estimate of 3.4 per 100 square meters from the first day's data, because of the larger numbers, although possible errors in the estimate of the rate of decline have been compounded. For the latter reason it is not desirable to go on to estimates from later days, although these are on the whole consistent. The estimates of density, per 100 square meters, of the wild population during the first two days of each of the four experiments, calculated as above, are given below.

Day	I	II	III	IV
1	3.4	8.3	8.5	8.2
2	4.2	8.5	6.4	7.5

Since the four experiments were conducted at different places and at different times, there is no necessity for agreement. There is indeed no necessity for agreement between different days in the same experiment since the wild population might conceivably receive a great addition or loss from one day to the next. It may be noted that if the estimated rate of decline of the released flies, 9.2 percent per day, does not apply on the first and second days, the above figures would be increased by 10 percent and 21 percent, respectively. It appears safe to conclude that the density of the wild population was about four per 100 square meters in experiment I and about twice as great in the other experiments.

THE DISPERSION OF THE RELEASED FLIES

The next question concerns the way in which the dispersion of the released flies takes place. The simplest hypothesis would be that the flies scatter at random to the same extent every day. If this holds, the variance of the whole population in a direction at right angles to any line through the point of release should rise by a uniform amount each day, according to the principle that the variance of the sum of independent contributions equals the sum of the separate variances. The variance of radial distances of flies from the point of release should also increase uniformly since it should be equal on any day to the sum of the variances in two directions at right angles to each other.

The averages of the observed variances along the lines of traps and the standard errors ($\sigma^2\sqrt{2/n}$) are given in tables 14 and 15. In every case the variance is more than twice as great on the second day than on the first and continues to increase, with exceptions on particular days. The irregularities in experiment I (actual but not significant decrease in variance on 4th, 5th and 9th days) are probably due to cessation of most activity on those days on which the temperature was 60° or less, in line with the interpretation of the low

TABLE 14

The variance ($\sigma^2=\Sigma r^2 f/n$) of the orange flies along a single line of traps, on successive days of each experiment. The origin is the point of release. The east-west and north-south lines are combined, and the central trap is counted twice. The unit of distance is that between stations (20 meters). The standard error, $\sigma^2\sqrt{2/n}$, is given.

DAY	I			II			III			IV		
	NO.	σ^2	SE	NO.	σ^2	SE	NO.	σ^2	SE	NO.	σ^2	E
1	279	3.8±0.3		584	8.7±0.5		674	8.4±0.5		1048	11.4±0.5	
2	609	8.1±0.5		354	21.0±1.6		532	22.2±1.4		—		
3	369	13.6±1.0		238	25.8±2.4		288	42.6±3.6		—		
4	171	13.0±1.4		276	34.5±2.9		166	41.6±4.6		—		
5	94	10.4±1.5		145	37.5±4.4		—			—		
6	215	17.8±1.7		—			—			—		
7	81	21.4±3.4		—			—			—		
8	69	32.4±5.5		—			—			—		
9	27	23.3±6.4		—			—			—		

TABLE 15

The variance of the distribution of orange flies along the two arms of the cross that were continued to the end of the experiment. The unit of distance is that between stations. The standard error is given.

DAY	NO.	II′ σ^2	SE	NO.	III′ σ^2	SE	NO.	IV′ σ^2	SE
1	337	7.6±	0.6	295	9.0±	0.7	635	11.3±	0.6
2	220	22.5±	2.1	236	30.5±	2.8	306	22.8±	1.9
3	128	22.7±	2.8	133	52.2±	6.4	102	46.5±	6.5
4	168	35.0±	3.8	69	40.3±	6.9	73	78.4±	13.0
5	90	35.7±	5.3	123	44.3±	5.7	51	73.1±	14.5
6	78	63.2±	10.1	58	73.0±	13.6		—	
7	79	64.5±	10.3	39	90.4±	20.5		—	

catches of both orange and wild flies on these days. But while there was no significant correlation between the size of catch and temperature above 60°, there is a significant correlation between the increment of variance and temperature. Excluding experiment I, this correlation was .75 based on 19 entries, and the regression of increment on temperature was 2.31±0.50 units (400m²) per degree Fahrenheit. Including experiment I, the correlation was .70. The regression was 1.17±.25. The regression is clearly not linear, there being relatively little dispersion below 70° in comparison with the large amount above.

Unfortunately the observed variance cannot be taken as representative of the variance of the whole population in an east-west or north-south direction because of the wide departures from normality in the first few days. We must return to the model of a distribution in which the ordinates at a given distance from the center in any direction are equal to the average catch at that distance from the point of release, in order to obtain less biased estimates. In such a distribution, the mean squared radial distance is $\int_0^{2\pi}\int_0^{\infty} r^3 dr d\theta / \int_0^{2\pi}\int_0^{\infty} r dr d\theta$. The expression $\Sigma r^3 \bar{f}/(\Sigma r \bar{f} + c/2\pi)$ may be used as an approximation. The estimates for each day in each experiment are given in table 16. These figures should be halved to give the variances of the population in one direction at right angles to a line through the center and thus most nearly comparable with the observed variances as given in tables 14 and 15. The latter, however, are very much less than half the corresponding entries in table 16 during the first days of each

TABLE 16

The estimated mean square distance from the point of release of orange flies on successive days of each experiment ($\Sigma r^3 \bar{f}/(\Sigma r \bar{f} + c/2\pi)$). *The figures under I, II, III and IV are based on all four arms of the cross, those under II', III', and IV' on the two arms that were continued to the end in each case. The unit of distance is that between trap stations (20 meters).*

DAY	I	II	II'	III	III'	IV	IV'
1	15.2	36.7	27.9	42.5	46.8	52.9	51.3
2	25.0	63.8	65.8	63.8	78.6	—	75.8
3	36.8	67.9	61.9	88.1	94.1	—	119.3
4	36.0	80.1	85.6	88.3	84.4	—	197.2
5	30.4	95.1	96.5	—	86.5	—	142.8
6	40.2	—	149.7	—	107.3		
7	43.6	—	127.2	—	142.4		
8	59.5						
9	52.5						

experiment when the forms of the distributions are farthest from normal. In the revised estimates the variance on the second day is on the average only 50 percent greater than on the first (I, II, III, IV') compared with 130 percent greater in the case of the observed variances. After the second day the variance rises at an approximately uniform rate (regression 4.51 ± 0.75 units for nine days) of experiment I, 16.80 ± 2.90 for seven days of experiment II, 13.75 ± 2.23 for seven days of experiment III, and 30.12 ± 11.13 for five days of experiment IV. The figure for experiment I is probably low because of low temperature. The unweighted average for the other three is 20.2

The correlation between the increment of variance and temperature is .47 based on 27 entries (regression $1.91 \pm .71$) and is not so great as in the original data. These are less reliable, however, than those based directly on the data. The revised estimates are designed to correct a systematic bias in the data as collected, but the variation in the increments become less reliable. The conclusions that seem warranted are that the flies scatter more widely on the first day than later but that there is a fairly uniform rate of dispersion after the

first day except as affected by temperature. The rate is relatively slow below 70° but increases greatly above this.

The actual average distances reached by the flies is a matter of interest. The average radial distance in the model adopted for the distribution is $\int_0^{2\pi}\int_0^{\infty} r^2 dr d\theta / \int_0^{2\pi}\int_0^{\infty} r dr d\theta$. The figures in table 17 were obtained by using the approximate formula $20\Sigma r^2\bar{f}/[\Sigma r\bar{f}+c/2\pi]$. It is to be noted that distance is

TABLE 17

The estimated average distance of orange flies from the point of release on successive days of each experiment. ($\Sigma r^2\bar{f}/(\Sigma r\bar{f}+c/2\pi)$). *The figures under I, II, III, IV are based on all four arms of the cross, those under II′, III′ and IV′ on the two arms that were continued to the ends of the experiments. The distances in this table are in meters.*

DAY	I	II	II′	III	III′	IV	IV′
1	59	98	85	101	107	118	114
2	81	135	136	138	158	—	144
3	104	139	128	167	179	—	189
4	103	156	158	167	163	—	243
5	95	167	165	—	169	—	211
6	111	—	213	—	196		
7	116	—	204	—	225		
8	141						
9	123						

here expressed in meters instead of station intervals. In experiments II, III, IV the flies reached average distances of more than 200 meters in a week or less. Individual flies were found at 220 meters from the point of release after one day in experiment I in spite of the low temperature (56°) on this day and the same or greater distance (up to 300 meters) after one day in the other experiments. The maximum distance reported was 500 meters on the third, fourth, and fifth days of experiment IV. Individual flies were found in or near the outermost traps in so many cases, as may be seen from tables 1 to 5, that it is extremely probable a few would have been found at still greater distances if the lines of traps had been extended indefinitely. Such flies would tend to increase the various estimates that have been made.

SECONDARY EXPERIMENTS

Most of the experiments made at Keen Camp in the summer of 1941 suffer from the disability that the experimental fields were so small that some of the flies passed beyond their confines in one day after the release. Only the middle portions of the resulting distribution curves, therefore, have been observed. Variances calculated from these data are evidently much lower than they should be on the basis of the 1942 data, and this regardless of whether orange-eyed flies or flies marked with nail polish are concerned. Only a single experiment, started on July 12, 1941, by the release of 1696 wild flies caught in the same locality and marked with nail polish, appears to be free from the above disability. On July 13, 70 marked flies were recaptured on a cross-shaped experimental field consisting of 45 traps. The variances observed on the two

axes are 6.2 ± 1.3 and 3.6 ± 0.8 in terms of the units used in tables 15 and 16 (namely, 400 m^2). The temperature was recorded only after the observed maximum of the activity of the flies, but by analogy with other temperature curves it should have been approximately 67°–68°F at the time of the maximum. Hence, the observed variances are somewhat lower than after one day in experiments II (8.7), III (8.4), and IV (11.4) at temperatures 70°, 70°, and 71°F, respectively, but higher than after one day in experiment I (3.8) at 56°F. On July 14, variances of 13.4 ± 3.0 and 17.7 ± 3.8 units were recorded. The temperature at the time of the maximum activity on that day was probably close to 69°F. The observed increment of variance is therefore of the right magnitude.

On July 16, 1942, 3493 orange-eyed flies were released at Pinon Flats. On July 17, 34 marked flies were recaptured on a cross-shaped field of 61 traps. This is an abnormally small proportion of recovery of marked flies, to be accounted for probably by the extreme aridity prevailing in the locality at that season. The variances on the two axes were 20.4 ± 4.1 and 23.0 ± 5.0 units. The temperature at the time of maximum activity was between 73° and 74°F. The observed variances are slightly higher than expected for the temperature, but not significantly so. It is possible that under unfavorable conditions the flies, seeking an escape, move more extensively than under favorable ones.

We have assumed throughout that the flies released at a given point spread uniformly in the surrounding territory. Yet, it is possible that the daily exposure of a series of baited traps might cause the flies to be drawn to the vicinity of the places where food is located. On a cross-shaped experimental field this would mean that the flies might move chiefly along the arms of the cross and not into the territory between the arms. The following test was arranged. The second of the four main experiments was run for seven days, and the numbers of the orange and of wild flies which were coming to the traps on the arms of the cross at different distances from the point of the release were therefore known (table 2). On June 24, 49 traps were exposed in the territory between the southern and the western arms of the cross. They attracted 1509 wild and 26 orange flies, which is about what is expected in a similar number of traps on the arms of the cross. Other evidence is provided by the numbers of wild flies captured on successive days on experimental fields. If flies were attracted to the traps from the surrounding territory and stayed near the traps till the next day, the numbers of flies caught on the experimental field would gradually increase with time. Our two years' experience shows that this is not so, although the numbers of flies visiting the traps on successive days are so greatly influenced by the temperature that the observations within any one experiment may be misleading (table 2–5).

In July and August of 1941 an attempt was made to depopulate a territory by systematic trapping and destruction of the flies within it. On a square shaped field 120×120 meters in size, 49 traps were set checkerboard fashion at 20 meters from each other. For 17 consecutive days all the flies which came to the traps on this field were caught and destroyed. About 300 meters away from the experimental field a control field with 10 traps was set; the flies

which came to these traps were counted and released at the end of the collecting time each evening. The flies caught on the experimental field on the second and the following days were either remnants of the original population which failed to enter the traps on the first day, or immigrants from the surrounding territory, or newly hatched ones within the territory. If the flies move only short distances, the ratios of the numbers of flies per trap on the experimental and the control fields should wane in the course of time. Furthermore, the numbers of flies in traps located close to the center of the experimental field should dwindle more rapidly than in traps at the periphery of the field. For about the first week, the figures seemed to indicate that the above expectations were on the way to fulfillment, but thereafter and till the end of the experiment the destruction of the flies had no effect whatever on the population density on the experimental field. This result, puzzling at the time, seems clear now. The experiment was started during relatively cool weather (62°–72°F at the time of maximum activity of the flies), but from the second week on weather turned warm (71°–78°, except on the 16th day). The distances traveled by the flies at temperatures of 70°F and up are so great that an experimental field 120×120 meters in size is not at all isolated from the surrounding territory. That wild flies move extensively at these temperatures has been shown also in another way. On July 19, 1941, a forest fire destroyed the vegetation, and presumably all the flies, in a large territory at Keen Camp. Eight days thereafter MR. H. LEWIS collected flies at a distance 260 meters inside from the margin of the burned area—the farthest distance tried.

AN EXPERIMENT ON DROSOPHILA MELANOGASTER

The migration rates found in *Drosophila pseudoobscura* appear to be of a greater order of magnitude than those recorded by the TIMOFEEFF-RESSOVSKYS (1940b, c) in *D. funebris* and *D. melanogaster*. Unfortunately, the TIMOFEEFF-RESSOVSKYS have published their data chiefly in the form of total numbers of flies found in each trap of an experimental field during the duration of the experiment; no temperature data have been published. However, figures 1 and 2 in the 1940c paper give the results of collecting on the fourth day of experiments involving *D. funebris* and *D. melanogaster*, respectively. The variances observed on the two axes drawn through the point of release on the *D. funebris* field turn out to be 127 and 44 m^2 (based on 11 and 9 flies). The average, 86 m^2 corresponds to only 0.21 of the unit used for *D. pseudoobscura*. This would indicate daily increment of variance 22 m^2, and hence an average distance after a day of only about 4 meters. The data for *D. melanogaster* show that the flies migrated entirely in one direction from the point of release—something never observed in *D. pseudoobscura* even in those (fortunately, very rare) instances when some days during an experiment happened to be windy ones.

On August 4, 1942, 3083 wild type *D. melanogaster* flies were released on the same cross-shaped field which had been used previously for the first experiment with *D. pseudoobscura* (p. 308). The flies were F_1 hybrids of two wild type strains raised in the laboratory. No mutant was used as a marker since the particular

experimental field chosen was free of an indigenous population of *D. melanogaster*. On August 5, 38 flies, and on August 6, 21 flies were recaptured. This is a smaller proportion of recapture of released flies than is customary in experiments with *D. pseudoobscura*. The variance on the first day was 256 m^2 (0.6 units) on one of the two axes (only two flies were recorded on the other axis outside of the point of release). On the second day the variance rose to 536 m^2 on the first and 118 m^2 on the second axis. The temperatures on both days declined from 77° to 68–67°F during the times when the flies were entering the traps. In view of the small number of the flies, it is difficult to tell the temperature at the time of maximum of their activity, although it was probably closer to the higher than to the lower of the two values just indicated. The daily increments of variance observed by us in *D. melanogaster* are about ten times as high as those found by the TIMOFEEFF-RESSOVSKYS in *D. funebris* (at temperatures which, however, might have been different), and about 20 times lower than found in *D. pseudoobscura* at similar temperatures.

The data are admittedly poor and not strictly comparable; nevertheless, they leave no doubt that the migration rates in different species of Drosophila are quite different. *D. funebris* and *D. melanogaster* are chiefly scavengers associated with man; *D. pseudoobscura* is rarely a scavenger. May the success of the two former species in adapting themselves to man-made environments be partly due to their relatively sedentary habits?

ESTIMATES FROM THE ALLELISM OF LETHALS

Attempts have been made in two preceding papers (DOBZHANSKY and WRIGHT 1941; WRIGHT, DOBZHANSKY, and HOVANITZ 1942) to estimate the effective size of breeding populations of *Drosophila pseudoobscura* on the basis of the frequency (p) of allelism of lethals in samples trapped at various distances apart. If the number of individuals interbreeding at random in each locality were sufficiently large, the frequency of each lethal would always be maintained at a certain equilibrium, as a consequence of opposed pressures of mutation and selection. The chance of allelism of lethals from the same local population would then be no greater than for lethals from remote populations. If, on the other hand, the number of individuals interbreeding at random is everywhere small, local differences would accumulate, causing a greater chance of allelism for pairs of lethals from the same locality than from distant localities. It turned out that in tests of 6294 pairs of lethals from regions so remote from each other that common origin is practically excluded, 26 or $0.41 \pm .08$ percent were found to be alleles. This was assumed to measure the chance of allelism due to independent mutation. For comparison with this, tests were made of 5537 pairs of lethals, which came from traps at relatively short distances apart. Of these 91 or 1.64 ± 0.17 percent proved to be alleles. Since the difference is highly significant, it was concluded that the random breeding unit is sufficiently limited in size to permit local variations in lethal frequency to exist.

It should be said that there is an alternative interpretation of this difference —namely, that the equilibrium frequencies themselves may vary from place

to place (most plausibly as a consequence of local differences in the action of selection on heterozygotes). The results of analysis of the data, however, were not favorable to this view.

The data could be broken down in various ways. In some cases the pairs of lethals came from the same "station" (a group of traps of which the extremes were not more than 100 meters apart). In others they came from different stations of the same "locality." Thus collections were made at two stations 200 meters apart at Wildrose in the Death Valley region, at two stations 200 meters apart in Andreas Canyon on Mt. San Jacinto, and at two stations 500 meters apart on Pinon Flats, Mt. San Jacinto. The locality Keen on Mt. San Jacinto was represented by five stations from 1 to 3.5 kilometers apart, sampling an area of about six square kilometers. This locality is of primary interest as that nearest and most similar to the locale of the dispersion experiments described in the present paper.

The above three localities on Mt. Jacinto were from 16 to 21 kilometers apart. All of the above studies were based on collections made in 1939. Collections made in 1937 in 11 isolated mountain forests in the Death Valley region about 320 kilometers from Mt. San Jacinto were analyzed in the 1941 paper. In each of these localities the flies were collected in lines of traps one-quarter mile to one mile long. No distinction was made in this case between flies from the same station and different stations.

The 1939 data could also be broken down according to whether the flies which furnished two tested lethals were captured within a month or at an interval of one to 11 months.

The results, subdivided roughly according to interval in space and time, are recapitulated below (table 18). The Death Valley data of 1937 (24 allelic pairs in 772 tests or 3.11 ± 0.63 percent) are included in the class of pairs collected within a month from stations 200 meters to 500 meters apart, although some undoubtedly came from the same trap and some from traps more than 500 meters apart.

It appears from these results that particular lethals may not only be present in excess in the population directly sampled at a station at a particular time but that this excess extends to some extent over considerable distances (more than a kilometer) and persists for several generations.

In interpreting these data the sampled population was assumed to consist of a certain effective number (N) of individuals. Immigration was assumed to replace this population to a certain extent (m) by flies in which the lethals are at their equilibrium frequencies. The total frequency of lethals in the populations studied was very much less than expected on the hypothesis that the mean frequencies are determined solely by mutation at the rate observed in the laboratory, balanced by elimination of the homozygotes appearing in a random breeding population. The deficiency could be due either to departures from random mating within the population under consideration measured by the inbreeding coefficient (F), or to selection against heterozygotes (rate s). Two extreme hypotheses with respect to s and F were tried. According to one $(s+F)$ is the same for all lethal mutations. According to the other, F is as-

sumed to be o in the populations sampled at stations, s is assumed to be so high for the great majority that are observed in the laboratory that they are very rarely found in nature, while s is assumed to be zero for those found in nature. The latter hypothesis, which increases considerably the estimated value of N, was not borne out by experiments in which lethal mutations were allowed to accumulate in heterozygotes for several generations before testing. In either case it was possible only to estimate N for given values of m.

In an area as large as that sampled at Keen (six square kilometers) it appears safe to assume that the total immigration per generation is a very small per-

TABLE 18

Recapitulation of all data on the frequency of allelism in Drosophila pseudoobscura in relation to distance in time and space.

	INTERVAL LESS THAN ONE MONTH				INTERVAL ONE TO 11 MONTHS				TOTAL			
DISTANCE BETWEEN TRAPS	NO. TESTS	SAME LOCUS NO.	%	S.E.	NO. TESTS	SAME LOCUS NO.	%	S.E.	NO. TESTS	SAME LOCUS NO.	%	S.E.
o to 0.1 km	777	16	2.06	±.51	1474	29	1.97	±.36	2251	45	2.00	±.30
0.2 to 0.5 km	1227	29	2.36	±.43	690	6	0.87	±.35	1917	35	1.83	±.31
Total o to 0.5 km	2004	45	2.25	±.33	2164	35	1.62	±.27	4168	80	1.92	±.21
1 to 3.5 km (Keen only)	466	6	1.29	±.52	903	5	0.55	±.25	1369	11	0.80	±.24
Total o to 3.5 km	2470	51	2.07	±.29	3067	40	1.30	±.20	5537	91	1.64	±.17
16 to 320 km									6294	26	0.41	±.08

centage, and effective m, measuring replacement by genes representative of the species with respect to lethal frequencies, would be still smaller. On taking the percentage of allelism (0.80±.24) at face value and assuming m=o, N comes out 20,000 by the first extreme assumption 104,000 by the other less favored assumption. If m is assumed to be .01, the estimate for N lies between 13,000 and 28,000. An estimate of 30,000 is as large as seems warranted, accepting 0.80 as the percentage of allelism. However, if the true value is less than 0.80 by the standard error (that is, p=0.56), the lower limit of the estimate for N rises to 53,000 for m=o; 34,000 for m=.01. On the other hand, a value larger than 0.80 is more probable than a smaller one, since most of the pairs of lethals from different stations at Keen were collected at intervals of a month or more. The percentage among those collected within the same month was in fact 1.29±0.52 percent but based on obviously inadequate numbers. If used, the lower limit of the estimate for N (with m=o) falls to 9000.

The estimate N=30,000 for the six square kilometers of Keen implies an effective density of 50 per 10,000 square meters, the approximate area sampled directly at a station. The percentage of allelism within stations at Keen was 2.45±0.49 percent. If N is taken as 50, m comes out about 0.50. These considerations were the basis for the final statement in the 1942 paper: "a typical station is estimated to have an effective N of only about fifty but with substantially no isolation from other stations in the locality." We believe that this is a valid deduction but the assignment of the definite value 0.50 to m is not

valid. The value 0.50 implies that deviations from equilibrium are swamped at a rate of 50 percent per generation. Obviously this would not permit any appreciable extension in space or persistence in time and is thus contrary to observation. There is in fact a certain minimum size of population, the "panmictic unit," below which the theoretical relation of m to N ceases to hold. The proper deduction is that the flies collected in traps at a station, while a direct sample of only about 10,000 square meters, are really a substantially random sample of flies from a much larger area. The 50 flies per 10,000 square meters is thus merely an estimate of effective density. The minimum interbreeding unit must be considerably larger than fifty.

THE PANMICTIC UNIT

It is of primary importance in dealing with a continuous population to determine the effective number of individuals in the panmictic unit, the population from which the parents of any given individual may be considered to be drawn at random (WRIGHT 1943). Associated with this is the question of the area occupied by this population. Take the emergence site of individuals as origin and consider the emergence sites of parents as forming a radially symmetrical bivariate normal distribution relative to this, defined by a certain standard deviation σ in either an east-west (x) or north-south (y) direction $z = e^{-(x^2+y^2)/2\sigma^2}/(2\pi\sigma^2) = e^{-r^2/2\sigma^2}/(2\pi\sigma^2)$. If the panmictic unit is taken as the effective breeding population of a circle of radius $c\sigma$, there are two types of error to be considered in assigning a value to c. On the one hand, if c is small, an appreciable portion of the parents are excluded. The proportion included is $\int_0^{2\pi}\int_0^{c\sigma} rz\,dr\,d\theta / \int_0^{2\pi}\int_0^{\infty} rz\,dr\,d\theta = (z_0 - z_{c\sigma})/z_0$ in which z_0 and $z_{c\sigma}$ are the ordinates at $r = 0$ and $r = c\sigma$. Effective N for the area inclosed by the circle of radius $c\sigma$ should be multiplied by $z_0/(z_0 - z_{c\sigma})$ to compensate for the excluded parents. On the other hand, the ratio of parents to total number decreases as r increases. Effective N should be multiplied by $\int_0^{2\pi}\int_0^{c\sigma} rz\,dr\,d\theta / \int_0^{2\pi}\int_0^{c\sigma} rz_0\,dr\,d\theta = 2(z_0 - z_{c\sigma})/c^2 z_0$ to correct for this. These two corrections compensate for each other if $c = \sqrt{2}$. As the mean square radial distance is $2\sigma^2$, effective N of the panmictic unit may be taken as equivalent to effective N of the population included in a circle, the radius of which is the square root of the mean square distance between emergence site of parent and of offspring.

In an actual circle of radius $\sqrt{2}\sigma$, the proportion 36.8 percent ($= z_{\sqrt{2}\sigma}/z_0$) of the parents of individuals at the center came originally from outside the circle. Considerably more than 50 percent of the parents of individuals near the margins came from outside. Thus there is about 50 percent replacement of the population of such a panmictic unit from the outside populations in each generation. Most of these immigrants, however, come from the immediate vicinity and may be expected to differ little in gene frequencies.

If q_i is the gene frequency of actual immigrants to the area in question, q_e that of the individuals in this area which they replace (not necessarily exactly the same as q, the mean gene frequency of the area), and q_t is the mean gene frequency of the species, the effective value of m is approximately $.50(q_e - q_i)/(q - q_t)$.

The stations at San Jacinto could be considered to be "panmictic" units only if $(q_e - q_i) = (q - q_t)$—that is, if the immediate immigrants are characterized by the gene frequencies of the species as a whole ($q_e = q$, $q_i = q_t$). Since there was clearly considerable extension of high frequencies of particular lethals to larger areas and considerable persistence, m must be considerably less than .50. If m is assumed to be .20, the percentage of allelism within stations at Keen implies a value of N of about 250 for the panmictic unit. Still accepting the estimated density of 50 per 10,000 square meters, this implies that the panmictic unit occupies some 50,000 square meters (radius 150 meters) and that there are 120 of them included in the area sampled at Keen. The smaller "localities" (Andreas, Pinon, most of those at Death Valley) would be only small multiples of the panmictic unit. This would at least partially account for the high average chance of allelism between stations of those localities. But swamping of exceptional frequencies at the rate implied by $m = .20$ is hardly compatible with the significantly high percentage of allelism between stations of the larger area of Keen. The values $m = .05$, $N = 1000$ would be more satisfactory. This implies an area of some 200,000 square meters (radius 250 meters) for the panmictic unit and the occurrence of about 30 in the locality of Keen.

If the panmictic unit is as large as this, it becomes a question whether one should not assume an appreciable value of m for Keen. If $m = .01$ for Keen, the minimum estimate for N falls to 13,000, and if $m = .02$, minimum N becomes 10,000. These suggest a value of N of about 15,000 for this locality, which implies an effective density of only 25 per 10,000 square meters. If these are correct, an estimate of effective N of the panmictic unit as small as 500 (but area 200,000 square meters as above) would be consistent with the observations.

These estimates are based on the chances of allelism within and between stations at Keen and include the data for collections made at intervals greater than a month as well as at shorter intervals. The best estimate for the chance of allelism within a panmictic unit is probably that obtained from stations 0 to 500 kilometers apart in all localities but restricted to tests of pairs collected within the same month. This figure is 2.25 ± 0.33. It yields the estimates $m = .05$, $N = 1100$ or $m = .10$, $N = 570$ (lower limits for the panmictic unit) in substantial agreement with those from Keen alone.

Summing up, rather extensive data indicating an excess chance of allelism of pairs of lethals from flies trapped 0 to 500 meters apart within the same month, coupled with evidence for partial persistence for longer periods and partial extension to considerably greater distances, lead to an estimate of the effective size of the panmictic unit of the order of 500 to 1000 individuals. The much less extensive data from flies trapped 1 to 3.5 kilometers apart indicate an effective density of the order 25 to 50 per 10,000 square meters. Combining these estimates gives some 200,000 square meters or a circle of radius of about 250 meters as a rough estimate of the area from which most of the parents of individuals are drawn. It is obviously highly desirable to be able to supplement these very indirect estimates by data on the actual density and the actual movements of the flies.

COMPARISON OF THE DEDUCTIONS FROM DISPERSION AND FREQUENCY OF ALLELISM

The investigations described in the present paper led to an estimate of about 400 flies per 10,000 square meters as the density of the wild population at Idyllwild at the beginning of June 1942 and of about 800 flies per 10,000 square meters from the middle of June to the end of July. The latter is 16 to 32 times the estimate of effective density based on frequency of allelism of lethals. But the direct estimates apply to the season of population maximum while the indirect estimate is theoretically the harmonic mean of the number of parents per generation throughout the year (that is,

$$1/N = \frac{1}{n} \sum_{i=1}^{n} (1/N_i)$$

for n generations per year and N_i breeding flies in the i'th generation). Effective N is thus much more closely related to the number of flies at population minimum (late winter at Keen and Idyllwild) than at population maximum. The estimates are consistent as far as present knowledge goes, but there is nothing in the estimate of summer density that forbids estimates of *effective* density several times larger or several times smaller than 25 to 50 per 10,000 square meters.

The amount of dispersion of the flies described in the present paper obviously bears on the area of the panmictic unit. There are, however, many uncertainties. The evidence indicates that the flies scatter more in warm than in cold weather. Thus the area from which parents are drawn may vary greatly from generation to generation, and the effective population number of this area may vary for this reason as well as from seasonal changes in population density. Full treatment requires a more complicated theory than has been developed. But populations that become thoroughly mixed in periods of great activity cannot drift apart much during the periods of low activity. It would appear that the unit area must be taken as that of which the radius is the square root of the mean square distance between emergence sites of parent and offspring at the period of greatest activity, although the effective population number is determined by the harmonic mean of the densities in this area in all generations (modified slightly by departures from panmixia due to splitting up in times of low activity, WRIGHT 1943). The marked deficiency in the number of lethals in comparison with the number expected on the basis of the observed mutation rate may be due more to inbreeding of this nature than to selection against the heterozygotes (see discussion WRIGHT, DOBZHANSKY, and HOVANITZ 1942, p. 359). This consideration favors acceptance of estimates of effective N close to those given by the minimal hypothesis (the assumption that $s=0$).

Another complication in relating the observed movements of the flies to the panmictic area comes from the likelihood of a difference in the mean square distance between emergence sites of father and offspring and that for mother and offspring. The former is compounded of the distance travelled by the male before encountering the female and that travelled by the female before laying the egg, which may be a considerable time, since females may carry sperm in

the spermatheca for weeks. The mortality curves and the curves of fertility in relation to time must also be taken into account in each case.

We will assume that the flies emerging at a given place scatter in a bivariate normal distribution with the mean square distance increasing by the same amount $2h^2$ each day. The variance of the distribution in one direction may be written h^2t where t is the number of days since emergence. The ordinate of the bivariate distribution is thus $z = e^{-r^2/2h^2t}/2\pi h^2t$. The ordinate of the distribution of distances is $2\pi rz$. But the population number is falling off according to some function of time, and productivity per individual is changing according to some other function of time. Let $\phi(t)$ be the product of these two functions and thus the total productivity in terms of time. In the absence of definite information of the situation in nature, assume that total productivity falls by a constant amount per day, reaching zero in k days, $\phi(t) = (1 - t/k)$. The mean square distance from emergence site of parent to offspring may then be represented by the following.

$$\overline{r^2} = \int_0^k \int_0^\infty r^3 z(1 - t/k)drdt \Big/ \int_0^k \int_0^\infty rz(1 - t/k)drdt.$$

This may easily be evaluated, noting that

$$\int_0^\infty r^3 zdr = 2h^2t \int_0^\infty rzdr = 2h^2t/2\pi.$$

$$\overline{r^2} = 2h^2k/3$$

It will be recalled that the distribution of the released flies in the experiments described here was far from the bivariate normal distribution assumed above at least on the first two days after release. There was excessive scattering of some of the flies on the first day, but after this the estimated mean square distance increased fairly uniformly. The average increment (experiments II, III, and IV) was 20.2 units per day. The conditions relating to newly emerged wild flies are so different from those relating to a large number of flies released at the same place that it seems fair to ignore the excessive dispersion of the latter on the first day and take the figure 20 units or 8000 m^2 as the best available estimate of $2h^2$.

No very satisfactory estimate of k is available. However it may be noted that the present data indicate that about 50 percent of a group of young flies disappear in the course of seven days. If productivity per fly is assumed to maintain the same level during this period but to fall off thereafter in such a way that total productivity continues with the same linear trend, k would be 14. The figure for males (including the period in which sperm are carried by the female) would presumably be greater. For $k = 14$, $\overline{r^2}$ is 37,300 square meters, giving 190 meters as the radius of the panmictic unit. If $k = 23$, the estimate of the radius rises to the 250 meters arrived at from consideration of the data on frequency of allelism. The estimates by the two methods are obviously in as close agreement as there is any right to expect.

The effective size of the panmictic unit in *Drosophila pseudoobscura* turns out

to be so large that but little permanent differentiation can be expected in a continuous population of this species owing to the genetic drift alone. It must, however, be pointed out that the figures obtained are valid only for the localities in which the experiments have been made and, of course, only for the species under study. Apart from species of Drosophila associated with man, *D. pseudoobscura* is the representative of the genus forming by far the densest and most widespread populations in at least the forested regions of the western United States. Furthermore, Keen Camp and Idyllwild are about as favorable localities for this species as could be found, and they were selected for experiments for just that reason. It is certain that the population densities in less favorable localities are much smaller than indicated above, and consequently the effective size of the panmictic unit arrived at is closer to the largest rather than to the smallest in this species. The panmictic units are probably smaller still in species that are less common or more restricted to habitats that recur only sporadically in space, especially if these species have lower dispersion rates than *D. pseudoobscura*. As shown above, *D. pseudoobscura* is certainly more mobile than *D. melanogaster* and *D. funebris*, the only two other species of the genus which have been examined in this respect.

SUMMARY

Four experiments are described in which an aggregate of 14,026 orange eyed *Drosophila pseudoobscura* were released at points near Idyllwild, California. Traps were set daily for five to nine days at intervals of 20 meters along lines crossing at right angles at the points of release (or along only one of these lines, greatly extended, in the later days), and captures of released and wild flies were recorded. Other experiments with *D. pseudoobscura* and ones with *D. melanogaster* are reported briefly.

The wild flies were uniformly distributed to the extent that some were captured in practically every trap (unless the temperature was below 60°F, under which the mean number per trap became small). However, there were marked irregularities due largely to a tendency to aggregate about old oaks and pines.

Most of the orange flies captured one day after release were still close to the point of release. Some, however, had scattered widely, causing the distribution along a line of traps to be markedly leptokurtic. Dispersion continued throughout the experiments, and the distributions became more nearly normal in the later days. Irregularities in the distributions were rather strongly correlated with the irregularities among the wild flies.

An attempt was made to estimate the relative numbers of wild flies and of orange flies that would have been captured on each day if traps had been distributed in a grid with 20 m spacing throughout the area reached by the orange flies. These estimates indicate that the orange population declined about 8 or 9 percent per day. The density of the wild population is estimated to have been 400 per 10,000 square meters in early June (1942) and 800 per 10,000 square meters in three experiments from mid-June to late July of the same year.

The dispersion of the flies was much affected by temperature, there being

very little dispersion below 60°F but increasing amounts with higher temperatures (regression 760 m^2 per degree). The mean squared distance travelled on the first day in three experiments in which the temperature was 70° or 71° was 17,600 m^2 (square root 133 meters). Dispersion increased by approximately equal increments of some 8000 m^2 in later days (average temperature 72°). The maximum distance recorded was 500 meters on the third (and later) days of one of the experiments.

The daily increment of the mean squared distance for *D. pseudoobscura* was more than 20 times that for *D. melanogaster* in the same locality. This in turn was nearly 10 times that observed by TIMOFEEFF-RESSOVSKY in the case of *D. funebris* in Berlin.

Analysis of population structure on the basis of the frequency of allelism of lethals in samples trapped at various distances apart had indicated an effective density of about 50 per 10,000 square meters (and reconsideration suggests reduction rather than increase). This is only one sixteenth of the density as estimated from the present data, but the latter applies to the population maximum of midsummer while effective density is more closely related to the population minimum than maximum (harmonic mean for all generations).

The data on allelism indicates that the parents of flies are typically drawn from a population of some 500 to 1000 individuals. The dispersion data indicate that the standard deviation of the distances between emergence sites of parent and offspring are of the order of 200 to 250 meters. This agrees as well as can be expected with the area of the "panmictic unit" deduced from the data on allelism.

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THE MORPHOLOGICAL MANIFESTATIONS OF A DOMINANT MUTATION IN MICE AFFECTING TAIL AND UROGENITAL SYSTEM

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THE study of hereditary variations has ceased to serve exclusively for the analysis of purely genetical problems. Students of other biological sciences have recognized for their own fields the value of the study of mutations. The embryologist, for example, has made use of hereditary malformations for the analysis of developmental problems. In the mammalian embryo it is usually difficult or impossible to interfere with developmental processes experimentally, but occasionally a mutation does what an experimenter is unable to do. In such a case, the investigator interested in developmental genetics may reverse the order of the experimental embryologist: he will start with a study of the results of the experiment of nature and proceed from there to an analysis of the events leading to these results.

The present work has followed this course with respect to a mutation which produces profound modifications in the posterior trunk region (lumbar, sacral and caudal spine, urogenital organs, intestinal tract) in the house mouse. This paper deals with the morphology of the mutants from birth to adult life, while a later paper will trace the embryological history of the malformations.

This *Sd* mutation has been described briefly in a previous publication (DUNN, GLUECKSOHN-SCHOENHEIMER, and BRYSON 1940). It was shown there that *Sd* segregates as a simple dominant to the normal condition and that it is genetically independent from a previously known dominant with similar effects on the tail (*T* = brachy). *Sd* is lethal when homozygous.

The heterozygotes are either tailless or have a short tail, depending on the residual heredity of the strain. The urogenital system is malformed in nearly all heterozygotes, one or both kidneys being absent or reduced in size. Due to the malformations of the urogenital system, the mortality of the heterozygotes after birth is very high. The homozygotes are born without any tail and with a very much shortened spinal column. Their kidneys are missing and they have a genuine closed cloaca and neither a urethral nor an anal opening. They die shortly after birth.

In this paper more detailed anatomical data both on the abnormalities of the spinal column and the urogenital system in the newborn homozygotes and in the newborn and adult heterozygotes are to be presented.

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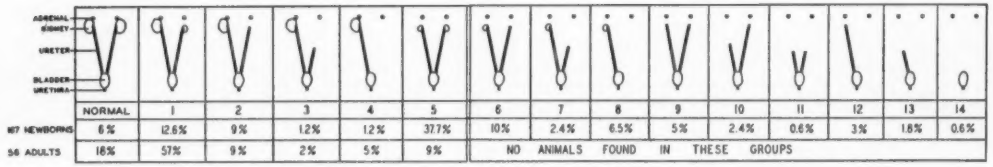

	NORMAL	1	2	3	4	5	6	7	8	9	10	11	12	13	14
167 NEWBORNS	6%	12.6%	9%	1.2%	1.2%	37.7%	10%	2.4%	6.5%	5%	2.4%	0.6%	3%	1.8%	0.6%
56 ADULTS	18%	57%	9%	2%	5%	9%	NO ANIMALS FOUND IN THESE GROUPS								

FIGURE 1. Distribution of kidney and ureter malformations in short-tailed and tailless heterozygotes (*Sd*+).

SKELETONS OF HETEROZYGOUS ADULTS

The malformations of the skeleton of the adult heterozygotes (*Sd*+) are concentrated in the posterior part of the spine. The number of thoracic vertebrae is normal, 13 usually being counted. Occasionally only 12 are found, but this number lies within the range of normal variation. The normal number of lumbars (six) is found in most cases also, but occasionally only five are present. Striking abnormalities are observed in the sacral region where the number of sacrals may vary between two and four (four being the normal number) with the third and fourth sacral vertebra frequently abnormally shaped, if present at all. Quite often the sacrals are fused abnormally with each other and with the caudals. The number of caudals is reduced considerably. As pointed out in a previous publication (DUNN et al. 1940), the tail length of the *Sd* heterozygotes varies with the genic environment. Accordingly, the number of caudal vertebrae varies. In addition to the reduction in number, the caudals show abnormalities in shape, and they are often fused with each other.

Aside from the malformations of the skeleton, the *Sd*-heterozygotes show striking abnormalities of the urogenital system.

UROGENITAL SYSTEM OF HETEROZYGOTES

Abnormalities of the urogenital system were detected in all but ten of the 167 newborn heterozygotes dissected and examined for internal malformations. The abnormalities ranged from complete absence of both kidneys and both ureters through all degrees of malformation to apparently normal urinary organs. In about 40 percent the renal vessels were reduced or absent. Such abnormalities occur only in connection with absence of one or both kidneys. Figure 1 shows the distribution of the abnormalities. The group consisting of animals with both kidneys present but reduced in size comprises the largest percentage of heterozygotes (37.7 percent). Reduction in size of one or both kidneys seems to be the commonest effect of this mutation, while complete absence is less frequent. It is not possible to decide whether the six percent classified as normals really are completely normal, since their kidneys were not studied histologically. The rest of the animals are distributed rather evenly among the remaining groups.

These severe urinary malformations appear not to have affected viability before birth, since the segregation ratio of normal and abnormal at birth was very close to that expected.

After birth the heterozygotes suffer a heavy mortality, and this is reflected in the disappearance before maturity of all the heterozygotes with the more severe kidney malformations. The 56 adult survivors which were dissected all fell into groups 1–5 (fig. 1). Only a few animals with two reduced kidneys survive. The bulk of the heterozygotes reaching maturity fall into the group of animals with one normal kidney and one reduced in size. About 16 percent belong to the groups with one normal kidney and one missing.

A comparison of the distribution of abnormalities among the newborns with that among the adult mice shows the importance of classifying the mice at

birth. The distribution picture obtained in mature mice differs very much from that obtained at birth, and a segregation ratio, based on adults, would be very abnormal indeed.

Malformations of the kidneys are more frequent on the left side of the body than on the right, both in newborns and adults. The frequency of left and right kidney malformations was determined in those cases where one kidney was either missing or affected. Out of 40 newborns, 31 had no kidney or a malformed kidney on the left side, while nine showed this condition on the right. Out of 41 adult mice with kidney abnormalities on one side only, 30 were abnormal on the left and 11 on the right. Both the differences in the newborns and adults are significant. This asymmetry in the distribution of kidney abnormalities was not observed by BAGG (1925), who, in studying another mutation affecting the kidneys, found 140 animals with the right kidney and 145 with the left kidney missing.

A preliminary examination of the histological structure of the affected kidneys shows these to be smaller but structurally the same as normal, at least superficially. The decision as to minute quantitative or qualitative differences in the structural elements of the normal and abnormal kidneys must be left to further more detailed histopathological studies.

In those cases where one kidney was missing in the adult mice, hypertrophy of the remaining kidney was observed. No abnormality was ever observed in the size or the position of the adrenals.

DISSECTIONS OF HOMOZYGOTES

While the condition of the urogenital system in the heterozygotes is such that at least part of them survive, the malformations in the homozygotes are so severe that none of them ever survives the first day after birth.

Ninety-two homozygotes were dissected after birth. Certain pathological features are common to all homozygotes, but others vary considerably in different individuals. The abnormalities regularly encountered, include:

(1) Complete absence of the tail.

(2) Spina bifida. In the lumbar and sacral region, the spinal cord is in parts unprotected by the vertebral column. This is due to the absence of all caudal or sacral vertebrae, or to the ventral clefts frequently found in the few lumbars present, and also to the fact that the spinal cord is not shortened but extends for its normal length. It therefore acquires abnormal shapes and often ends in a cyst which may be found anywhere in the sacral region, sometimes directly underneath the dorsal epidermis.

(3) Absence of rectum and anal opening. The intestine never continued beyond the colon in any of the homozygotes dissected. An imperforate anus was regularly found.

(4) Absence of urethral opening. A urethral opening never was found, not even in those cases where there was a small genital papilla.

(5) Absence of kidneys in their normal position. In none of the homozygotes was either of the kidneys ever found in its normal position.

(6) The displaced kidneys found in dissections (see below) were always extremely abnormal in size and structure.

(7) A regularly occurring genuine closed cloaca—that is, junction of intestinal and urogenital structures without any excretory duct.

(8) Ascites—that is, presence of fluid in the abdominal cavity, due to the abnormalities of the urogenital and intestinal organs.

The variable features are:

(1) In many cases the lumbars are completely absent; occasionally one to three malformed lumbars may be found.

(2) Due to the shortening of the vertebral column, abnormalities such as luxated hind limbs are encountered occasionally.

(3) The genital papilla may be either completely absent or reduced in size.

(4) The abnormalities of the intestine may include stenosis or atresia of the colon descendens, aside from the regular absence of the rectum. In the cases with a stenotic or atretic colon, the intestine usually acquires its normal shape again in the most caudal part of the colon descendens, just before joining the urogenital organs.

(5) A very small solitary kidney may be found in a median position on the level of the cloaca representing a joint remnant of the two normal kidneys shifted caudad.

(6) Rudiments of the ureters may be present leading from the solitary kidney remnant to the cloaca.

(7) The bladder may be present or not. It is only rarely of normal size, usually it is quite reduced, and frequently the point of junction of the urinary and the genital ducts and the colon is represented by a small vesicle only.

(8) A considerably shortened urethra may sometimes be found in those cases where the bladder is present in normal or nearly normal size.

(9) Occasionally, a malformation of the genital ducts is observed in the female: one or both uterine horns may be found to be stenotic.

In summarizing the results of dissections of hetero- and homozygotes we may say that in both cases, *Sd* has a strong effect on the posterior spine region and the urogenital system. The malformations of the homozygotes are more extreme than those of the heterozygotes and thus lead to the death of all homozygotes, while the malformations of the heterozygotes lead to the death of a fairly large number of animals, thus making *Sd* a dominant semi-lethal. The malformations of the axial skeleton and those of the urogenital system are closely connected with each other and inherited as a unit. Seventy-eight newborns with normal tails from crosses of $Sd+ \times Sd+$ or $Sd+ \times ++$ were dissected as controls, and their urogenital systems were found to be normal.

DESCRIPTION OF ANIMALS HETEROZYGOUS FOR TWO SHORT-TAIL MUTATIONS ($Sd+$; $T+$)

As reported previously (DUNN et al. 1940), the two tail shortening mutations *Sd* and *T* have a cumulative effect, the double heterozygote of *Sd* and *T* being tailless always. Studies of the axial skeleton of $Sd+$ $T+$ adults revealed more

extreme malformations than were found in *Sd*+ mice: only five lumbar vertebrae are present, and they are abnormally shaped and fused with each other. The sacrum consists of two or three strikingly abnormal vertebrae or rudiments of vertebrae; these are fused with each other, and frequently their single elements are hardly recognizable. Sometimes no caudal vertebrae at all, but occasionally two or three of them are found. Kyphoses and lordoses of the spine in the cervical, thoracic, or lumbar regions are very common. The urogenital system in these mice closely resembles that of the simple *Sd* heterozygotes.

DISCUSSION

The syndrome described represents the only known case of simple inheritance of so severe a malformation in mammals. We are dealing here with a dominant semilethal, since about 70 percent of all heterozygotes die between birth and weaning (DUNN et al. 1940).

Although the external phenotype of animals heterozygous or homozygous for *Sd* resembles that of other known short-tailed or tailless mutants in mice very closely, it differs from them not only in the mode of inheritance, but also in two other respects: *Sd* is characterized by its simultaneous effect on the axial skeleton and the urogenital system; furthermore, studies of the embryonic development of the short-tailed and the tailless phenotype have revealed that the malformations of the axial skeleton of the *Sd* heterozygotes and homozygotes are due to pathological processes different from those leading to the abnormalities of the other tail mutants investigated in mice. The malformations in the tail of *Sd* mice are not due to primary abnormalities of the notochord, as was found to be the case in the mutations at the *T* locus (*cf.* CHESLEY 1935; GLUECKSOHN-SCHOENHEIMER 1938), but to disturbances of vascularization. The results of the embryological investigations will be reported in a separate paper.

The phenotypic expression of the effect of the *Sd* mutation on the tail is similar to that of *T* (Brachyury) described by CHESLEY (1935); it is in most cases not possible to tell *T*+ and *Sd*+ animals apart as far as their external characteristics are concerned. Still, *Sd* and *T* turned out to be not only different genes, but not even alleles, and are located on different chromosomes. Thus, the same abnormality in structure of a particular organ may be brought about by different genes, each of these in turn affecting different organs at the same time: thus *T* affects somites, allantois, etc. (*cf.* CHESLEY, 1935), while *Sd* affects the urogenital system and intestine. It is possible to combine these two genes and let their effects become cumulative: as mentioned before the double heterozygotes are always tailless. Tail and urogenital characters are inherited as a unit in *Sd* mice, abnormalities of the urogenital system never having been found without simultaneous malformations of the tail.

It is not possible to decide whether the effect of *Sd* on the axial skeleton, the urogenital system and the intestinal tract is due to one cause responsible for the abnormalities of all three organ systems or whether axial skeleton, urogenital system, and intestinal tract are affected separately without any causal con-

nections between the individual effects. Developmental studies indicate an early effect of *Sd* on the vascular system, and it may well be that such a disturbance of vascularization may in turn produce abnormalities in all posterior body structures. If this turns out to be the case the syndrome produced by *Sd* could be traced back to one single effect of the gene. But failure to find such a common cause responsible for all ensuing malformations does not necessarily prove that several different processes are affected by *Sd*; it merely puts off the answer to the question.

The developmental studies of this material should reveal more about the interrelationships during the development of the different parts of the posterior trunk region.

The hereditary malformations described here remind one of similar conditions found sporadically in human and animal pathology; whereas in mice and rats, abnormalities of this sort with a definite genetic background have been reported.

The absence of one or both kidneys, as found in the heterozygous *Sd* mice has been reported in man, in descendants of X-rayed mice, and in rats. Nothing is known about the possible familial incidence of this malformation in human beings (GRUBER 1927).

Sporadic cases of malformations of the urogenital system and the intestinal tract as severe as those found in the homozygous *Sd* mice have been described in human pathology also (*cf.* GRUBER). When dealing with malformations of this kind in man, it seems well to remember the simple hereditary mechanism underlying the abnormalities in the *Sd* mice.

No abnormalities as severe as those reported here have previously been described in animals, with the exception of the most extreme cases of stub homozygotes in rats (RATCLIFFE and KING 1941).

Kidney malformations occurring in descendants of a group of X-rayed mice, found in connection with eye and foot abnormalities, have been reported by BAGG (1925). These abnormalities are definitely inherited, the mode of inheritance approaching the behavior of a mendelian recessive, and are considered by BAGG to be manifestations of a general tendency to abnormal structure. BAGG describes the absence of kidneys as well as abnormalities such as reduction in size, hydronephrotic and polycystic kidneys. While the reduced kidneys resemble those found in *Sd*+ mice, hydronephrosis and polycystic kidneys were never observed in the *Sd* strain.

It seems interesting to note that a higher incidence of kidney malformations on the left side—as found in our material—has been reported for unilateral kidney abnormalities in man, also. While the figures for right and left do not differ significantly in man, they do point in the same direction as do our data. BAGG (1929) and BONNEVIE (1934) mention asymmetries favoring the left side for the foot abnormalities in the abnormal Little and Bagg mice. The distribution of kidney abnormalities in BAGG's mice, however, is completely symmetric: 140 on the left to 145 on the right. Asymmetries in the expression of morphological abnormalities are well known and have been extensively discussed by LANDAUER (1939).

A hereditary malformation in rats, due to a simple recessive called stub, affecting among other body structures tail and urogenital system, has been described by RATCLIFFE and KING (1941). The phenotype of the homozygotes is quite variable, resembling to a certain extent both the *Sd* heterozygotes and homozygotes, depending upon the degree of expression. A closer resemblance to its syndrome of effects has been found in a mutation in mice in our laboratory recently and will be described in a later paper.

SUMMARY

The morphological effects of the dominant mutation *Sd* on axial skeleton and urogenital system are described in detail. *Sd* is completely lethal when homozygous and acts as a dominant semi-lethal when heterozygous.

Lumbar, sacral, and caudal regions of the spine are abnormal: vertebrae in these regions may be deleted, abnormally shaped, or fused with each other. The malformations of the axial skeleton in the homozygotes are more extreme than those in the heterozygotes.

Reduction of kidney size and absence of one or both kidneys are the most common features of the effect of *Sd* on the urogenital system in the heterozygote.

In the homozygotes, in addition to complete absence of kidneys, the posterior part of the intestine is abnormal, a genuine closed cloaca being found.

The effects of *Sd* and another dominant tail mutation *T* are cumulative.

Comparison of this mutant with hereditary and sporadic cases with similar symptoms in rats, mice, and man shows that *Sd* is unique in being at the same time a simply segregating single mutation, and the cause of more or less constant syndromes of severe abnormalities in spine, urogenital system, and gut.

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